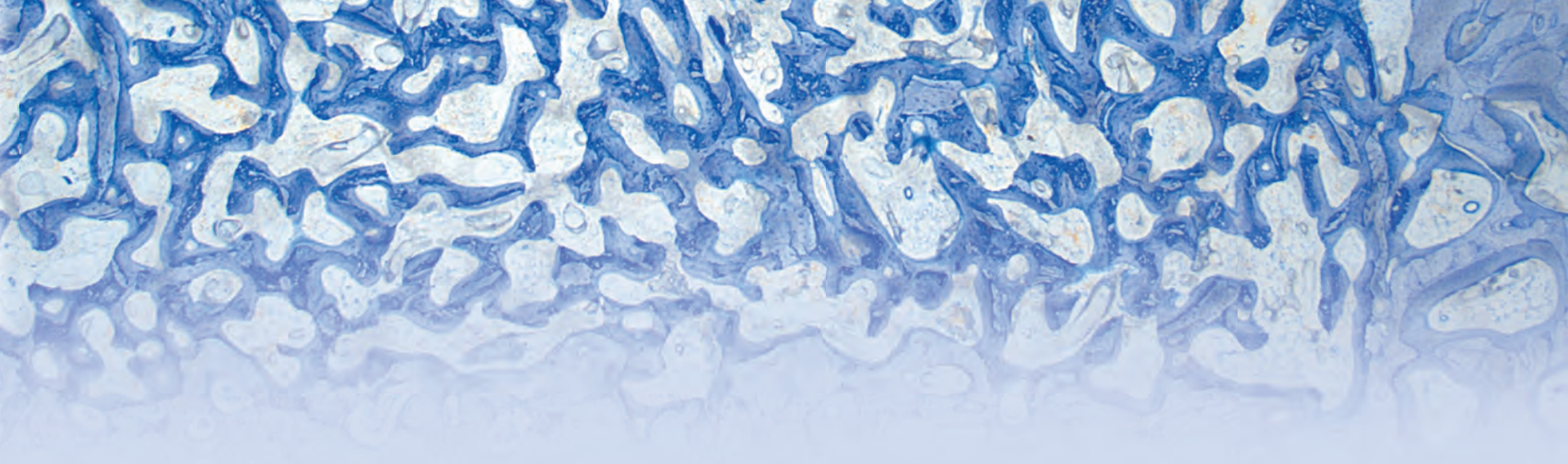
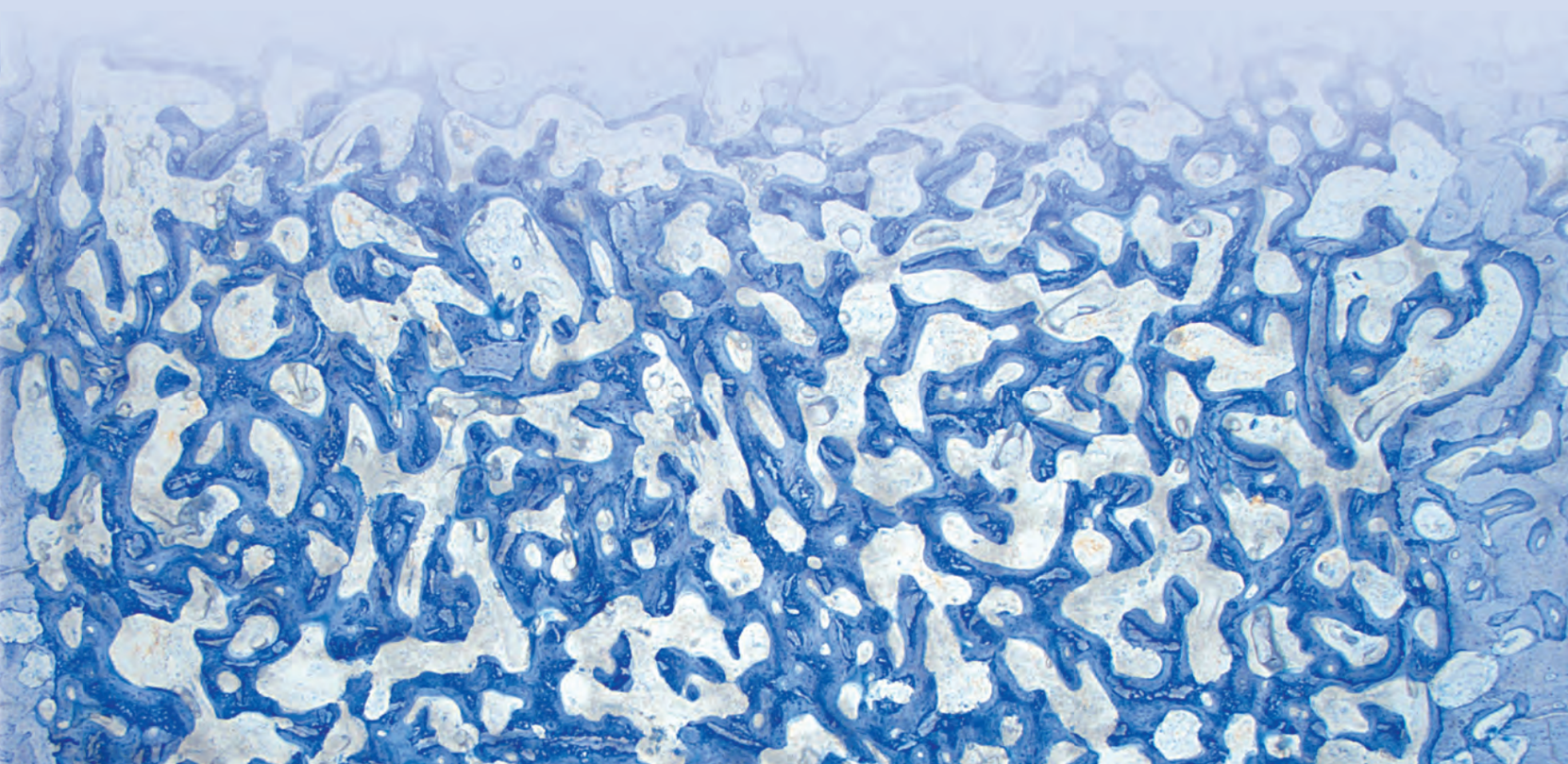


30 Years of Guided Bone Regeneration

THIRD EDITION

Edited by

Daniel Buser, DDS, Prof em Dr med dent



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Daniel Buser, DDS, Prof em Dr med dent
Professor Emeritus
School of Dental Medicine
University of Bern
Bern, Switzerland

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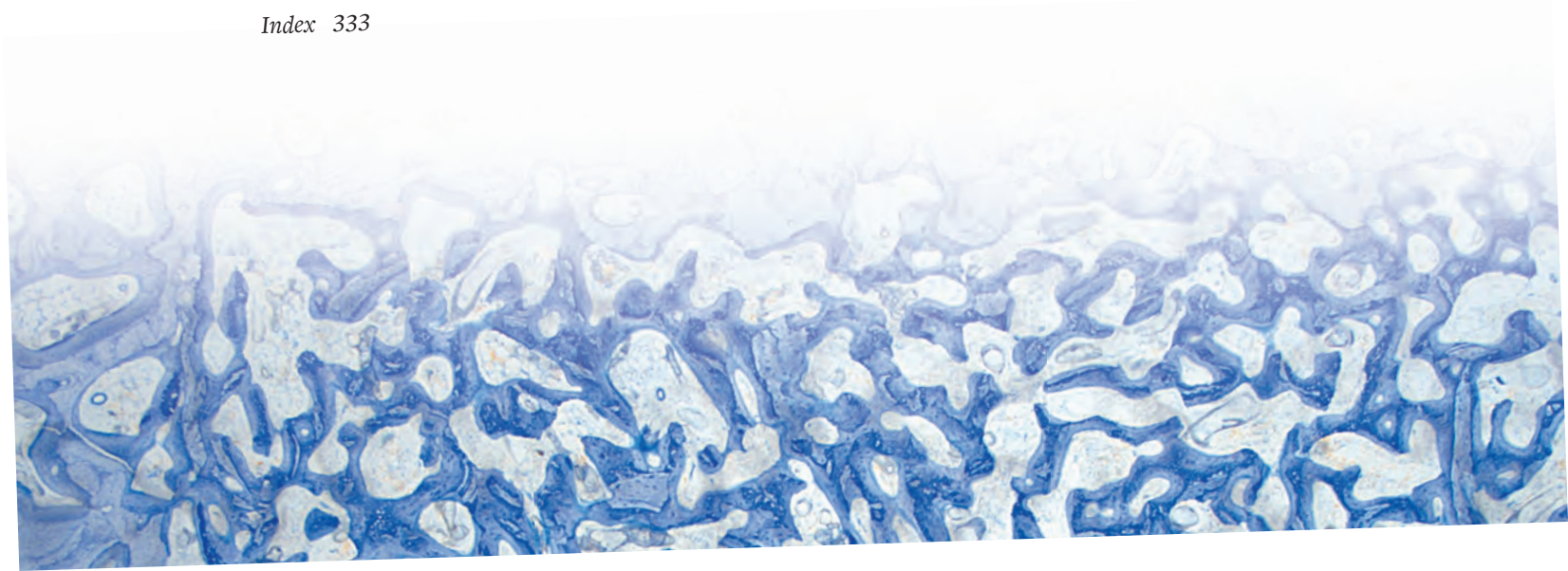

Foreword

There have already been three decades of scientific documentation and successful clinical experience in the field of GBR—a truly impressive accomplishment! In this third edition of an already well-established textbook, authored and edited under the judicious leadership of Professor Danny Buser, a carefully selected international panel of experts has updated and shed light from all relevant angles on one of the most significant recent achievements of contemporary dental medicine. The text not only surveys 30 years of progress made; it also comprehensively defines the current state of the art in GBR and its tremendous impact, namely on implant dentistry. Clinical protocols aimed at reducing overall treatment complexity and time, as well as diminishing patient morbidity, have been developed and refined during recent years. In addition, based on the remarkable levels of reliability and predictability of GBR, numerous new avenues for clinical application have been opened.

In fact, the knowledge of which techniques and associated biomaterials are recommended today, linked to the indispensable robust scientific documentation, provide the clinician with the basis for target-oriented clinical decision making in view of the subsequent treatment. This includes the consideration of the practitioner's individual state of education and competence. Namely, the SAC concept—which objectively differentiates straightforward, advanced, and complex cases in relation to the difficulty level of a given clinical situation—is of particular importance and has been strongly promoted by the main author for many years.

The current third edition of a textbook that has twice already previously reached the status of a true standard of reference has clearly outperformed its two predecessor issues. Beyond any doubt, oral surgeons, periodontists, prosthodontists, and general practitioners, as well as dental students, will find all the detailed information relevant to successful implementation of GBR in daily practice, ultimately to the benefit of countless patients.

Urs C. Belser, DMD, Prof em Dr med dent
Professor Emeritus
School of Dental Medicine
University of Geneva
Geneva, Switzerland

Dedication

This textbook is dedicated to Robert K. Schenk, Prof Dr med, who was Professor of Anatomy at the University of Bern, Switzerland. He was a world-renowned scientist in the field of bone physiology and bone healing. His instruction on the basics of bone healing was what allowed for the tremendous progress with GBR we made in the 1990s. Dr Schenk's chapter on the basics of bone healing in the first GBR book was a sensation at that time. He was able to illustrate his knowledge with fantastic histologic pictures produced by his lab. Besides his generosity to share his knowledge and wisdom, he was a true friend and mentor.

Robert K. Schenk, Prof Dr med (1923–2011)

Preface

The utilization of barrier membranes for the regeneration of bone defects has significantly changed implant dentistry in the past 30 years and clearly expanded the utilization of dental implants in patients. This principle is called guided bone regeneration (GBR or GBR technique), and was first described in 1959 by Hurley and colleagues for the treatment of experimental spinal fusion. In the 1960s, the research teams of Bassett and Boyne tested Millipore filters for the healing of cortical defects in long bones and osseous facial reconstruction, respectively. The authors utilized these filters to establish a suitable environment for osteogenesis by excluding fibrous connective tissue cells from bone defects. However, these studies did not lead to a clinical application of barrier membranes in patients at that time.

The clinical potential of barrier membranes was picked up in the early 1980s in the field of periodontology by the research team of Nyman and Karring, who systematically examined barrier membranes for periodontal regeneration. A few years later, barrier membranes were also tested for the regeneration of bone defects in experimental studies. The first three studies were done in Gothenburg by Dahlin and Nyman. Based on promising results in these studies, clinical testing of barrier membranes began in implant patients in the late 1980s. After 5 years of intensive experimental and clinical work, the first edition of the textbook *Guided Bone Regeneration in Implant Dentistry* was published in 1994, and it received a high interest by readers in the field of implant dentistry. In 2009, the second edition of the GBR book was published with an update of the scientific knowledge and the surgical techniques being utilized after 20 years of a wide clinical application of GBR.

In the past 12 years, the scientific knowledge and the clinical experience have evolved further. During these years, many fine-tuning efforts have been made for the various surgical techniques to improve the regenerative outcomes, or to reduce the surgical invasiveness for patients. Therefore, it was time to make a new effort to once again analyze the scientific basis of the GBR technique and its clinical applications. The result is in your hands, the third edition of the GBR book, called *30 Years of Guided Bone Regeneration in Implant Dentistry*. This book is again written for the surgical clinician with an interest and experience in implant dentistry.

As an introduction to the topic of the book, chapter 1 discusses the development and fine-tuning phase of the GBR technique over the past 30 years. Chapter 2 covers the biologic basis of bone regeneration and presents a scientific update on bone formation and bone remodeling. The excellent histology utilizing nondecalcified sections is based on more than 30 years of experimental research, and it presents the details of bone regeneration in general and the details of bone formation in membrane-protected defects with bone grafts or bone substitutes in particular. Chapter 3 is completely new and describes the molecular and cellular characteristics of autogenous bone chips, and how they release various growth factors when put in a mixture of blood and physiologic and sterile saline. Chapter 4 is also completely new and describes the hard and soft tissue alterations following tooth extraction. Clinicians need to understand these biologic mechanisms for proper selection of the most suitable treatment option in postextraction implant placement. Chapter 5 is also new and systematically describes the surgical and anatomical factors influencing the regenerative outcome of GBR procedures, including the interesting classifications of defect morphology.

In the clinical section of the book, chapters 6 to 14, clinical procedures associated with different indications of the GBR technique are presented in detail. Each chapter deals with specific indications and describes the criteria for patient selection, the step-by-step surgical procedure, and aspects of postoperative treatment. Emphasis is given to incision technique and flap design; the selection, handling, and placement of barrier membranes; the combination of membranes with autogenous bone grafts and low-substitution bone fillers; and aspects of wound closure. These chapters of the book reflect the immense progress and excellent documentation of GBR in the past 10 to 15 years, and its outstanding importance in daily practice of implant therapy.

Acknowledgments

As editor, I cordially thank all authors and coauthors for their great effort and time to realize this textbook. It has been very intensive work during a pandemic crisis, but a satisfying experience to collaborate with colleagues of such international reputation and high quality. Some of them are long-term personal friends, which makes the pleasure even greater. I also want to share that all authors, including myself, agreed to have the authors' royalties entirely paid into the Buser Implant Foundation, a foundation established in August 2019 right after my retirement as Professor and Chairman at the Department of Oral Surgery and Stomatology, University of Bern, after 20 years of service. The foundation's objectives are the promotion of education and research in the field of implant dentistry by providing personal stipends and junior investigator grants to young colleagues of our profession. The first Buser Foundation Scholarship in Implant Dentistry has been awarded in spring 2021.

I also thank Bernadette Rawyler, who created all the beautiful digital artwork in my chapters. These illustrations have made it much easier to communicate the correct messages and necessary information from the authors to the reader.

Last but not least, I also cordially thank Bryn Grisham and Marieke Zaffron of Quintessence Publishing for their excellent collaboration to realize this book. The quality work and the quality printing of Quintessence was again superb and is highly appreciated. It reflects almost 30 years of close collaboration with Quintessence Publishing, both in Berlin and in Chicago. I thank Horst Wolfgang Haase, Christian W. Haase, as well as Alexander Ammann for this excellent collaboration over so many years, which was based on trust, respect, and friendship.

Contributors

Mauricio G. Araújo, DDS, MSc, PhD
Head of the Research Group in Periodontics and Implant Dentistry
Department of Dentistry
State University of Maringá
Maringá, Brazil

Thomas von Arx, DDS, Prof Dr med dent
Deputy Clinic Director
Department of Oral Surgery and Stomatology
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Maria B. Asparuhova, PhD
Research Group Leader
Laboratory of Oral Cell Biology
Dental Research Center
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Urs C. Belser, DMD, Prof em Dr med dent
Professor Emeritus
School of Dental Medicine
University of Geneva
Geneva, Switzerland

Dieter D. Bosshardt, MSc, PhD
Professor
Robert K. Schenk Laboratory of Oral Histology
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Vedrana Braut, DDS, Dr med dent
Research Collaborator
Department of Oral Surgery and Stomatology
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Private Practice
Matulji, Croatia

Daniel Buser, DDS, Prof em Dr med dent
Professor Emeritus
School of Dental Medicine
University of Bern
Bern, Switzerland

Vivianne Chappuis, DDS, Dr med dent
Professor
Department of Oral Surgery and Stomatology
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Stephen T. Chen, MDSc, PhD
Clinical Associate Professor
Department of Periodontics
Melbourne Dental School
The University of Melbourne
Parkville, Australia

Francesco D'Aiuto, DMD, M Clin Dent, PhD
Professor and Chair
Periodontology Unit
Eastman Dental Institute
University College London
London, United Kingdom

Adam Hamilton, BDSc, DCD
Director of the Division of Regenerative and Implant Sciences
Department of Restorative Dentistry and Biomaterials Sciences
School of Dental Medicine
Harvard University
Cambridge, Massachusetts

Simone F. M. Janner, DDS, PD Dr med dent
Senior Physician
Department of Oral Surgery and Stomatology
School of Dental Medicine
Faculty of Medicine
University of Bern
Bern, Switzerland

Simon S. Jensen, DDS, Dr odont
Professor
Section of Oral Biology and Immunopathology
Department of Odontology
Faculty of Health and Medical Sciences
University of Copenhagen

Professor
Department of Oral and Maxillofacial Surgery
Centre of Head and Orthopedics
Copenhagen University Hospital
Copenhagen, Denmark

Sascha A. Jovanovic, DDS, MS
Founder and Academic Chairman
gIDE Institute
Los Angeles, California

Alberto Monje, DDS, MS, PhD
Adjunct Clinical Assistant Professor
Department of Periodontics and Oral Medicine
School of Dentistry
University of Michigan
Ann Arbor, Michigan

Assistant Lecturer
Department of Periodontology
Faculty of Dentistry
Universitat Internacional de Catalunya
Barcelona, Spain

Federico Moreno, Lic Odont, M Clin Dent
Clinical Lecturer
Periodontology Unit
Eastman Dental Institute
University College London

Private Practice
London, United Kingdom

Ausra Ramanauskaite, DDS, Dr med dent, PhD
Assistant Professor
Department of Oral Surgery and Implantology
Faculty of Medicine
Goethe University
Center for Dentistry and Oral Medicine (Carolinum)
Frankfurt, Germany

Isabella Rocchietta, DDS, MSc
Honorary Senior Research Associate
Periodontology Unit
Eastman Dental Institute
University College London

Private Practice
London, United Kingdom

Frank Schwarz, Prof Dr med dent
Professor and Chairman
Department of Oral Surgery and Implantology
Faculty of Medicine
Goethe University
Center for Dentistry and Oral Medicine (Carolinum)
Frankfurt, Germany

Istvan Urban, DMD, MD, PhD
Assistant Professor
Department of Restorative Dentistry
School of Dentistry
Loma Linda University
Loma Linda, California

Associate Professor
Department of Periodontics and Oral Medicine
School of Dentistry
University of Michigan
Ann Arbor, Michigan

1

The Development of Guided Bone Regeneration Over the Past 30 Years

Daniel Buser, DDS, Prof em Dr med dent

Modern implant dentistry based on the concept of osseointegration recently celebrated its 50th birthday.[1] The tremendous progress made in the rehabilitation of fully and partially edentulous patients is based on fundamental experimental studies performed by two research teams. One team was located in Sweden and headed by Prof P-I Brånemark from the University of Gothenburg; the other was located in Switzerland and headed by Prof André Schroeder from the University of Bern. In the late 1960s and 1970s, the two research groups independently published landmark papers describing the phenomenon of osseointegrated titanium implants.[2–4] An *osseointegrated implant* was characterized by direct apposition of living bone to the implant surface.[5–7]

In the early phase of this development, several prerequisites were identified for osseointegration to be achieved.[2,3] Some of these have been revised over the past 50 years; others are still considered important. In order to achieve osseointegration, the implant must be placed using a low-trauma surgical technique to avoid overheating the bone during preparation of a precise implant bed, and the implant must be inserted with sufficient primary stability.[5,8] When these clinical guidelines are followed, successful osseointegration will predictably occur for nonsubmerged titanium implants (single-stage procedure) as well as for submerged titanium implants (two-stage procedure), as demonstrated in comparative experimental studies.[9,10]

When clinical testing of osseointegrated implants first began, the majority of treated patients were fully edentulous. Promising results were reported in retrospective studies.[11–13] Encouraged, clinicians increasingly began using osseointegrated implants in partially edentulous patients, and the first reports on this utilization were published in the late 1980s and early 1990s with promising short-term results by various groups.[14–18] As a consequence, single-tooth gaps and distal extension situations have become more and more common indications for implant therapy in daily practice. Today, these practices dominate in many clinical centers.[19–21]

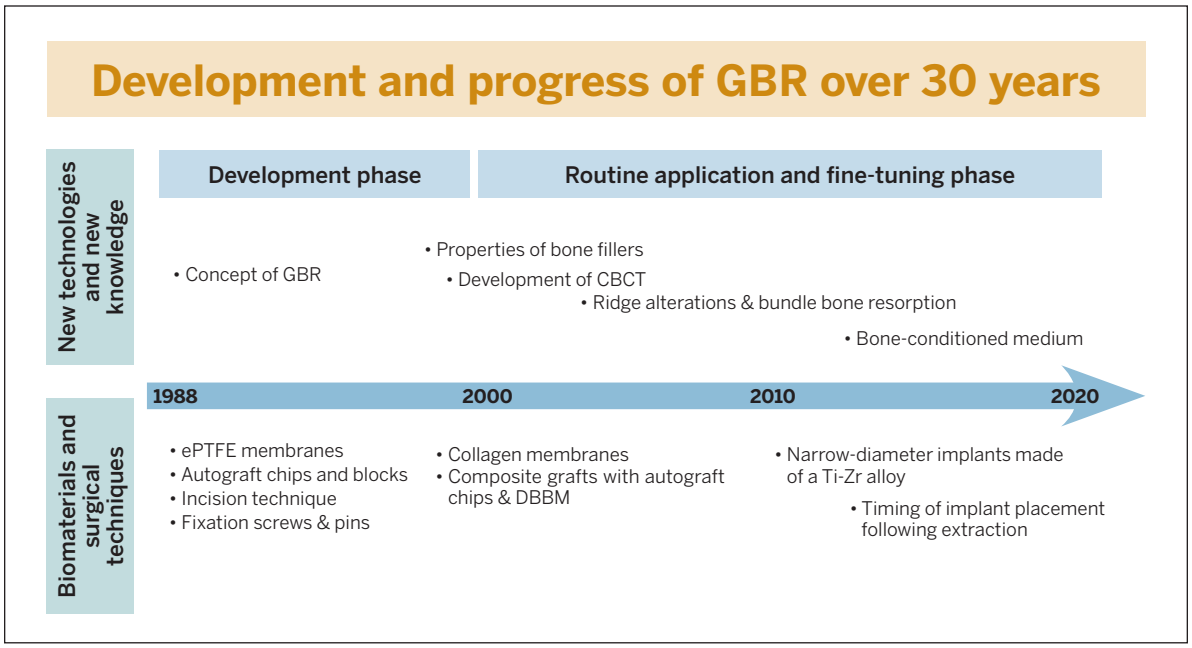

Fig 1-1 Development of GBR over 30 years since the late 1980s. ePTFE, expanded polytetrafluoroethylene; DBBM, deproteinized bovine bone mineral; Ti-Zr, titanium-zirconium.

One of the most important prerequisites for achieving and maintaining successful osseointegration is the presence of a sufficient volume of healthy bone at the recipient site. This includes not only sufficient bone height to allow the placement of an implant of adequate length, but also a ridge with sufficient crest width. Clinical studies in the 1980s and 1990s showed that osseointegrated implants lacking a buccal bone wall at the time of implant placement had an increased rate of soft tissue complications and/or a compromised long-term prognosis.[22,23] To avoid increased rates of implant complications and failures, these studies suggested that potential implant recipient sites with insufficient bone volume should either be considered local contraindications for implant placement or should be locally augmented with an appropriate surgical procedure to regenerate the local bone deficiency.

During these early decades, several attempts were made to develop new surgical techniques to augment local bone deficiencies in the alveolar ridge in order to overcome these local contraindications for implant therapy. The proposed techniques included vertical ridge augmentation using autogenous block grafts from the iliac crest in extremely atrophic arches,[24,25] sinus floor elevation procedures in the maxilla,[26–28] the application of autogenous onlay grafts for lateral ridge augmentation,[29–31] or split-crest techniques such as alveolar extension plasty.[32–34]

During the same period, in addition to these new surgical techniques, the concept of guided bone regeneration (GBR) with barrier membranes was introduced. Based on case reports and short-term clinical studies, various authors reported first results with this membrane technique for the regeneration of localized bone defects in implant patients.[35–40]

This textbook will provide an update on the biologic basis of the GBR technique and its various clinical applications for implant patients. Clinical experience with GBR in daily practice now spans 30 years. These 30 years can be divided into a development phase and a phase of routine application with extensive efforts to fine-tune the surgical procedure (Fig 1-1). The focus was on improving the surgical technique, expanding the range of applications, improving the predictability for successful outcomes, and reducing morbidity and pain for the patients.

Development Phase of GBR

The use of barrier membranes for implant patients was certainly triggered by the clinical application of barrier membranes for periodontal regeneration, called *guided tissue regeneration* (GTR). GTR was first developed in the early 1980s by the group led by Nyman et al.[41,42] The initial studies were performed with Millipore filters, which had already been used in experimental studies in the late 1950s and 1960s for the regeneration of bone defects.[43–45] However, these studies had no impact on the development of new surgical techniques to regenerate localized defects in the jaws, because the potential of this membrane application was probably not recognized at that time.

The two papers by Nyman et al[41,42] in the field of GTR, both of which demonstrated successful treatment outcomes of GTR procedures, were received with great interest and led to increased research activities in the mid to late 1980s.[46–49] These studies were already being performed with expanded polytetrafluoroethylene (ePTFE), which is a bioinert membrane and became the standard membrane for GTR and GBR procedures during the development phase of both techniques. The use of ePTFE membranes for bone regeneration was initiated in the mid 1980s by the group of Dahlin et al, who performed a series of preclinical studies.[50–52] These studies confirmed the concept that the application of an ePTFE membrane established a physical barrier that separated the tissues and cells that could potentially participate in the wound healing events inside the secluded space. The barrier membrane promoted the proliferation of angiogenic and osteogenic cells from the marrow space into the bone defect without interference by fibroblasts. These events were nicely demonstrated by Schenk et al[53] in a landmark experimental study in foxhounds. The current biologic understanding of wound healing events in membrane-protected bone defects is presented in detail in chapter 2 of this textbook.

The use of ePTFE membranes for GBR procedures started in the late 1980s. The main objective was to achieve regeneration in peri-implant bone defects in implant sites with local bone deficiencies. The GBR technique has been used with both simultaneous and staged approaches. Implant placement with simultaneous GBR was predominantly used for immediate implant placement in postextraction sites to regenerate peri-implant bone defects[35,36,38] or for implants in sites with crestal dehiscence defects.[40] The staged approach was used in clinical situations with healed ridges but an insufficient crest width. The membrane technique was used to enlarge the crest width with a first surgery, and implant placement took place after 6 to 9 months of healing in a second surgical procedure.[37]

Early on, several complications were observed with both approaches, and modifications of the surgical techniques were proposed to improve the predictability of successful treatment outcomes. One frequent complication was the collapse of the ePTFE membranes, which reduced the volume of the regenerated tissue underneath the membrane. In addition, some of the regenerated sites demonstrated insufficient bone formation and the formation of a periosteum-like tissue underneath the membrane.[37,40] Therefore, bone fillers such as autografts or allografts were recommended by various groups, primarily to support the membrane and reduce the risk of membrane collapse.[54–56] The combination of ePTFE membranes and autogenous bone grafts provided good clinical outcomes for both approaches. Some of these patients are still being followed and documented up to 25 years after surgery (Figs 1-2 to 1-4).

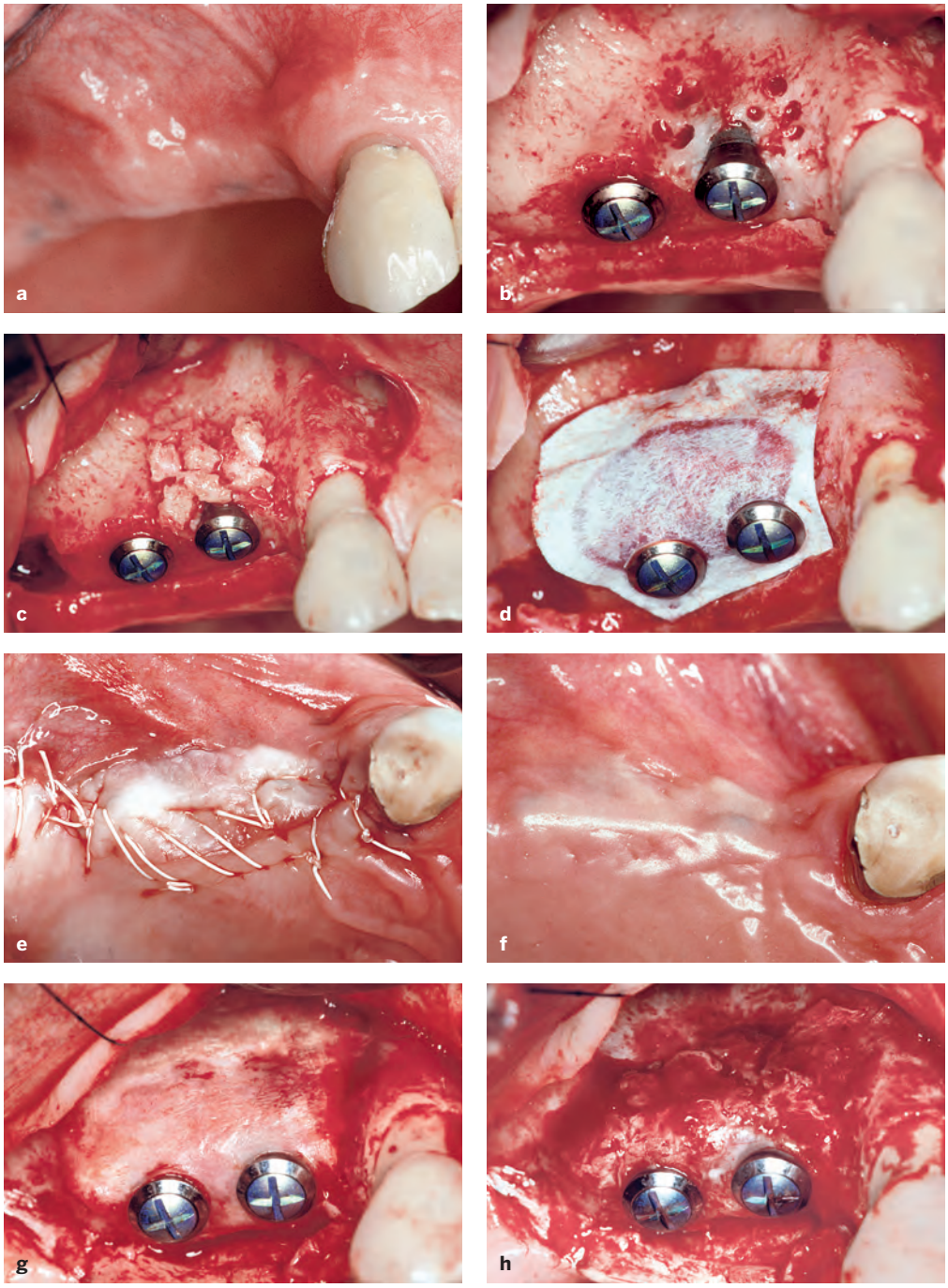

Fig 1-2 Case 1. *(a)* Preoperative status (1991). Distal extension situation in the right maxilla of a man with a healed ridge. Two titanium implants were planned to allow a fixed prosthesis. *(b)* Both implants were placed, resulting in a crestal dehiscence defect at the mesial implant. The cortical bone surface was perforated with a small round bur to open the marrow cavity and stimulate bleeding in the defect area. *(c)* Locally harvested bone chips were applied to support the ePTFE membrane and to stimulate new bone formation in the defect area. *(d)* A bioinert ePTFE membrane was applied to function as a physical barrier. The punched membrane was stabilized around the necks of both implants. *(e)* Following incision of the periosteum, the surgery was completed with a tension-free primary wound closure. *(f)* Clinical status 4 months after implant surgery. The wound healing was uneventful. *(g)* Reopening after 4 months of healing. A second surgery was necessary to remove the nonresorbable membrane. *(h)* The clinical status following membrane removal showed successful bone regeneration in the defect area at both implants. →

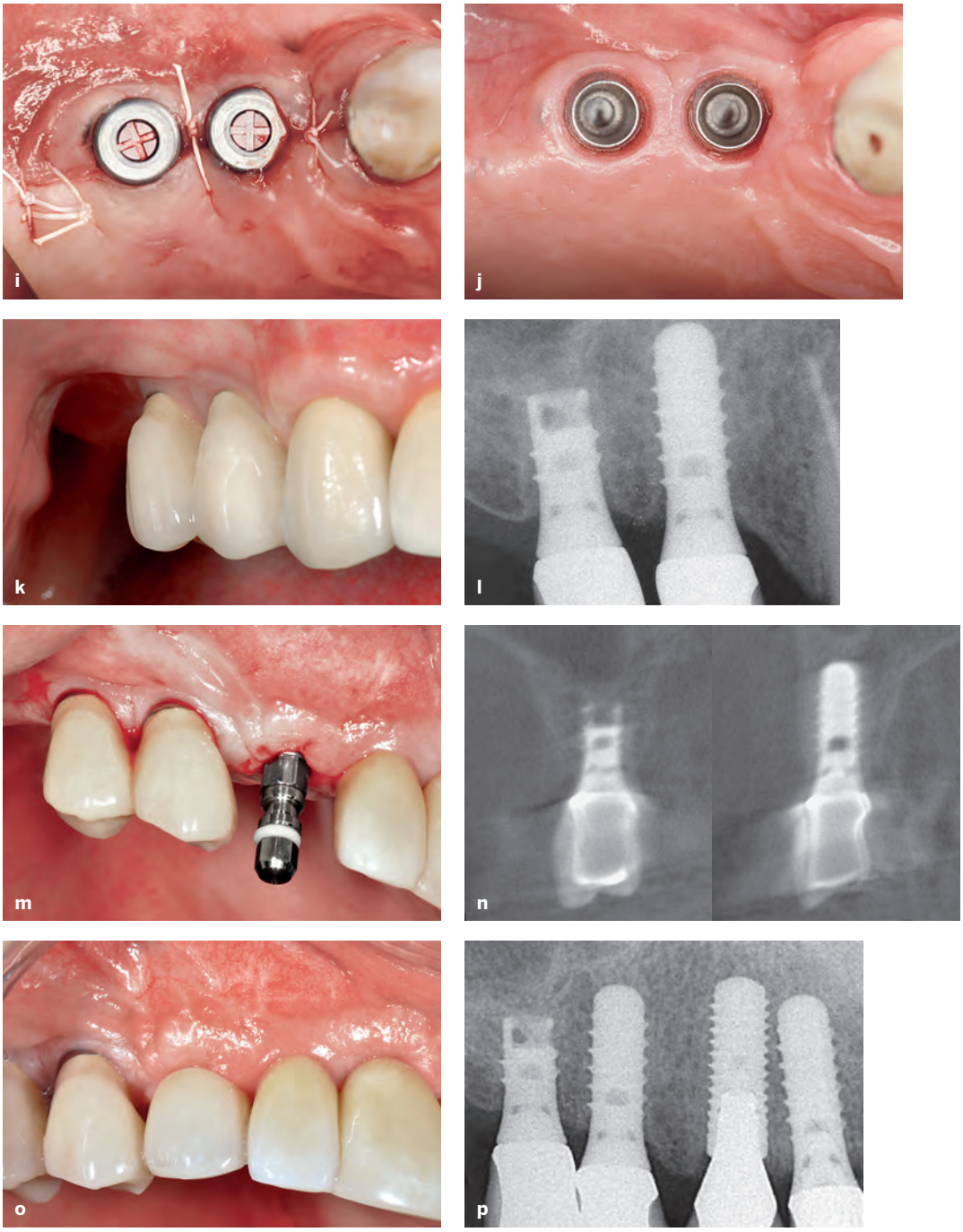

Fig 1-2 Case 1. *(cont) (i)* Longer healing caps were applied, and the soft tissue margins were adapted and secured in place with interrupted sutures. *(j)* Two weeks later, the soft tissues had healed, and both implants could be restored with a single crown. *(k)* The clinical status at the 15-year follow-up examination (2006) showed a satisfactory treatment outcome with stable peri-implant soft tissues. *(l)* Radiographic follow-up at 15 years: The bone crest levels were stable around both implants, which are splinted. *(m)* In 2010 (19 years after the initial surgery), an additional implant was placed in the canine site as late implant placement with a flapless approach. The clinical view during surgery showed stable peri-implant soft tissue at both implants in the premolar sites. *(n)* During perioperative examination of the canine implant site, a CBCT scan was taken. The orofacial cuts showed a thick facial bone wall for both premolar implants, which had been in function for 19 years at the time. *(o)* Clinical status after completion of the new single crown at the canine site. The treatment outcome was very satisfactory considering when the GBR procedure was done (1991). *(p)* Periapical radiograph after completion of therapy. The two tissue-level implants in the premolar sites had been in function for 19 years, and both showed stable peri-implant bone crest levels. This was the final follow-up examination, as the patient sadly developed dementia and passed away a few years later.

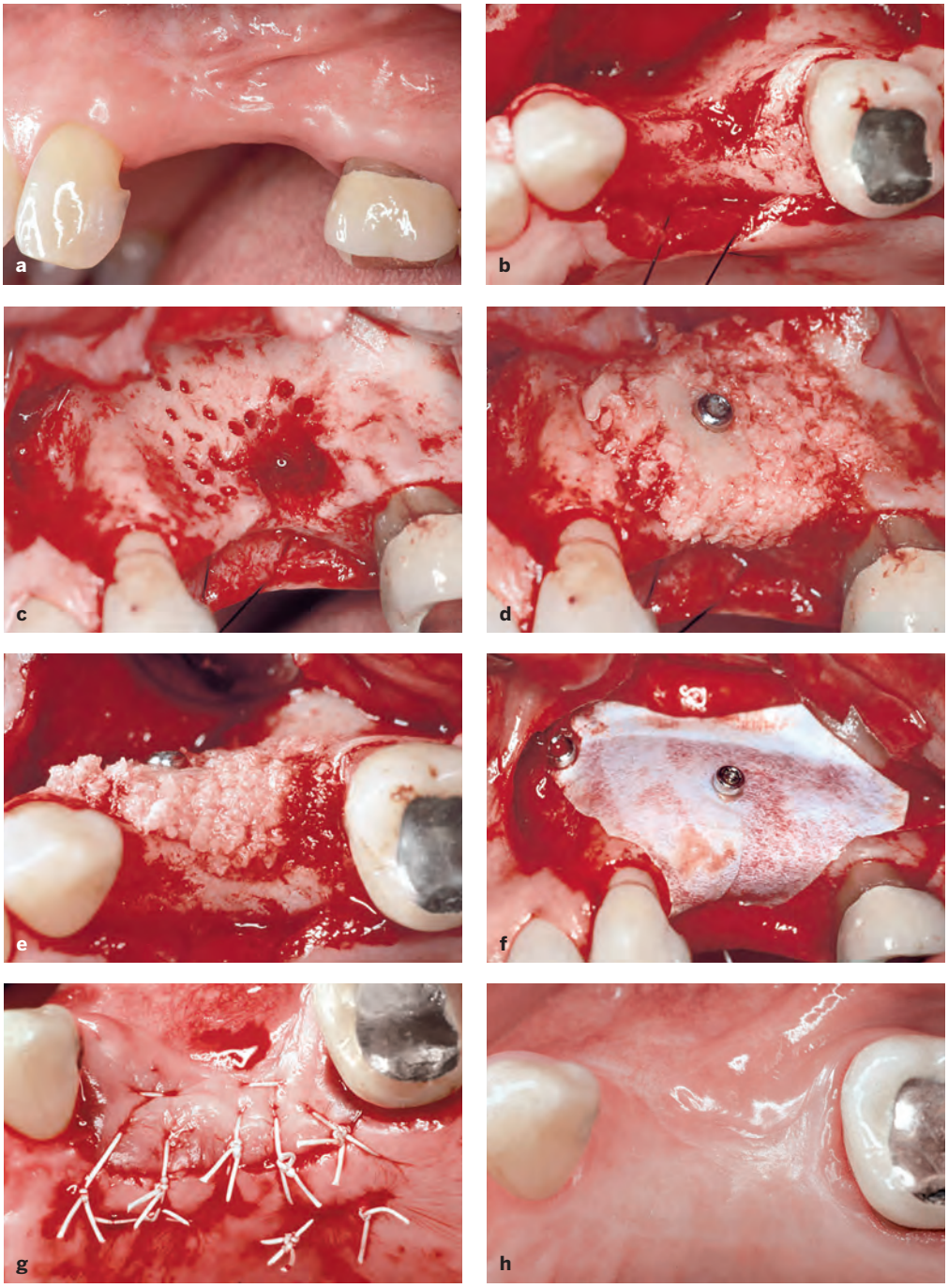

Fig 1-3 Case 2. *(a)* Preoperative view (1994). The buccal view of this woman's left maxilla shows two missing premolars. The buccal aspect is flattened. *(b)* The occlusal view during surgery shows a significant buccal flattening and a buccal bone defect in the area of the second premolar. *(c)* Prior to block application, the entire buccal bone surface was perforated to open the marrow cavity. The bone defect was debrided from scar tissues. *(d)* An autogenous block graft harvested from the chin was applied and fixed with a fixation screw. Bone chips were used to augment the entire surrounding area. *(e)* The occlusal view shows the volume of the augmented ridge. *(f)* Buccal view of the applied ePTFE membrane to cover the augmented ridge as a bioinert barrier membrane. *(g)* Primary wound closure was achieved with several mattress and interrupted single sutures using 4-0 and 5-0 ePTFE sutures. *(h)* Six months after ridge augmentation, the clinical status shows healthy soft tissues following a healing period free from complications. →

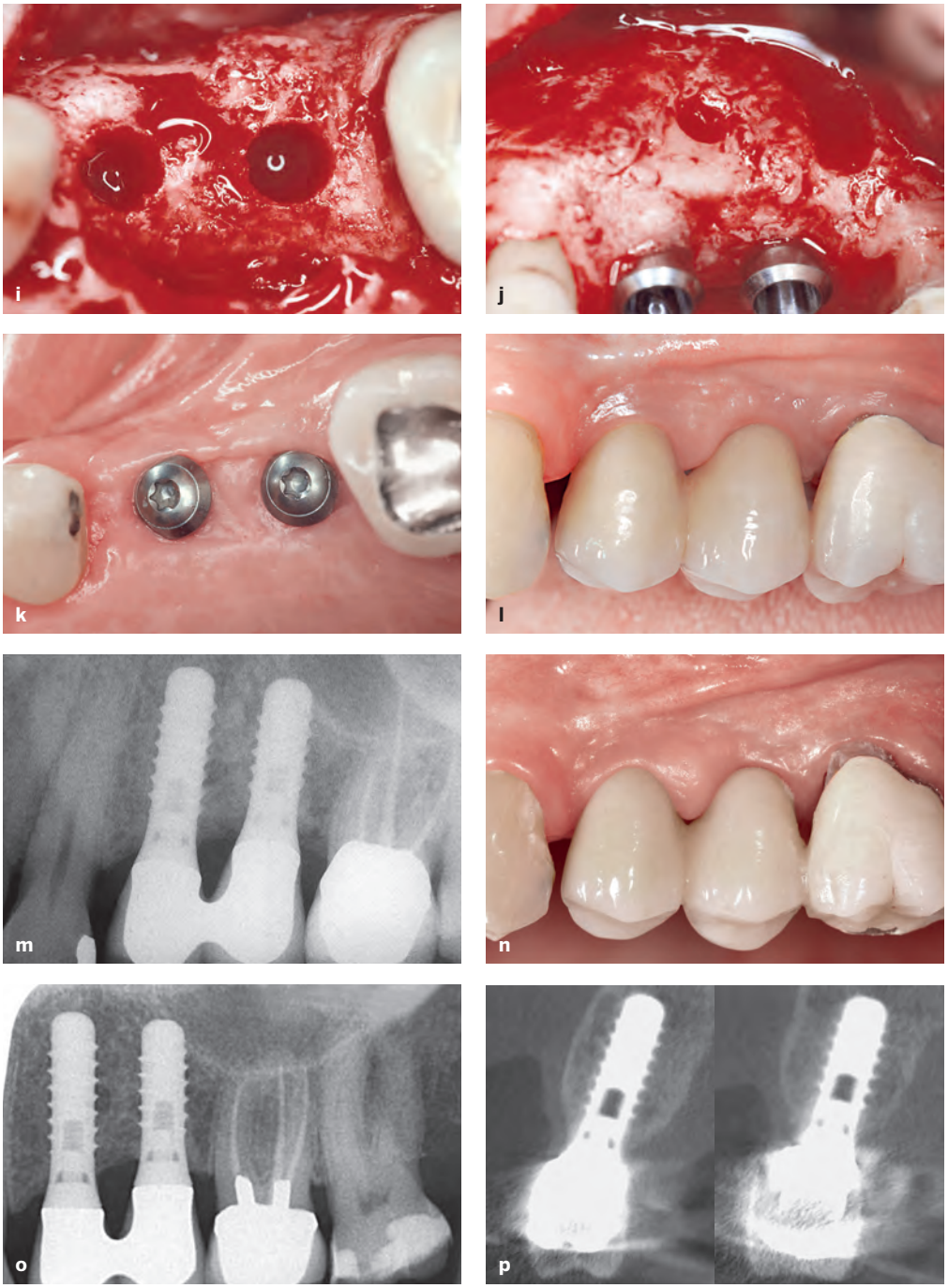

Fig 1-3 Case 2. *(cont) (i)* Following flap elevation and membrane removal, the occlusal view demonstrates an excellent ridge volume and thick buccal bone wall following implant bed preparation. *(j)* The buccal view confirms successful ridge augmentation. The block graft can still be recognized, and it is covered in some areas with newly formed bone. *(k)* Clinical status following 3 months of nonsubmerged healing for both implants. The peri-implant mucosa was healthy and included a nice band of keratinized mucosa. *(l)* Clinical status at the 10-year examination (2005) shows the two splinted implant crowns. The peri-implant mucosa was stable with no signs of a peri-implant pathology. *(m)* The periapical radiograph at the 10-year examination confirms stable bone crest levels around the two tissue-level implants with a hybrid design. *(n)* The 25-year follow-up examination (2019) shows the clinical status with quite healthy peri-implant mucosa, although the plaque control is no longer perfect in this elderly patient (age 86). *(o)* The periapical radiograph confirms stable bone crest levels at both tissue-level implants. *(p)* The CBCT scan shows fully intact, thick buccal bone walls for the implants in the first premolar (left) and second premolar (right) sites.

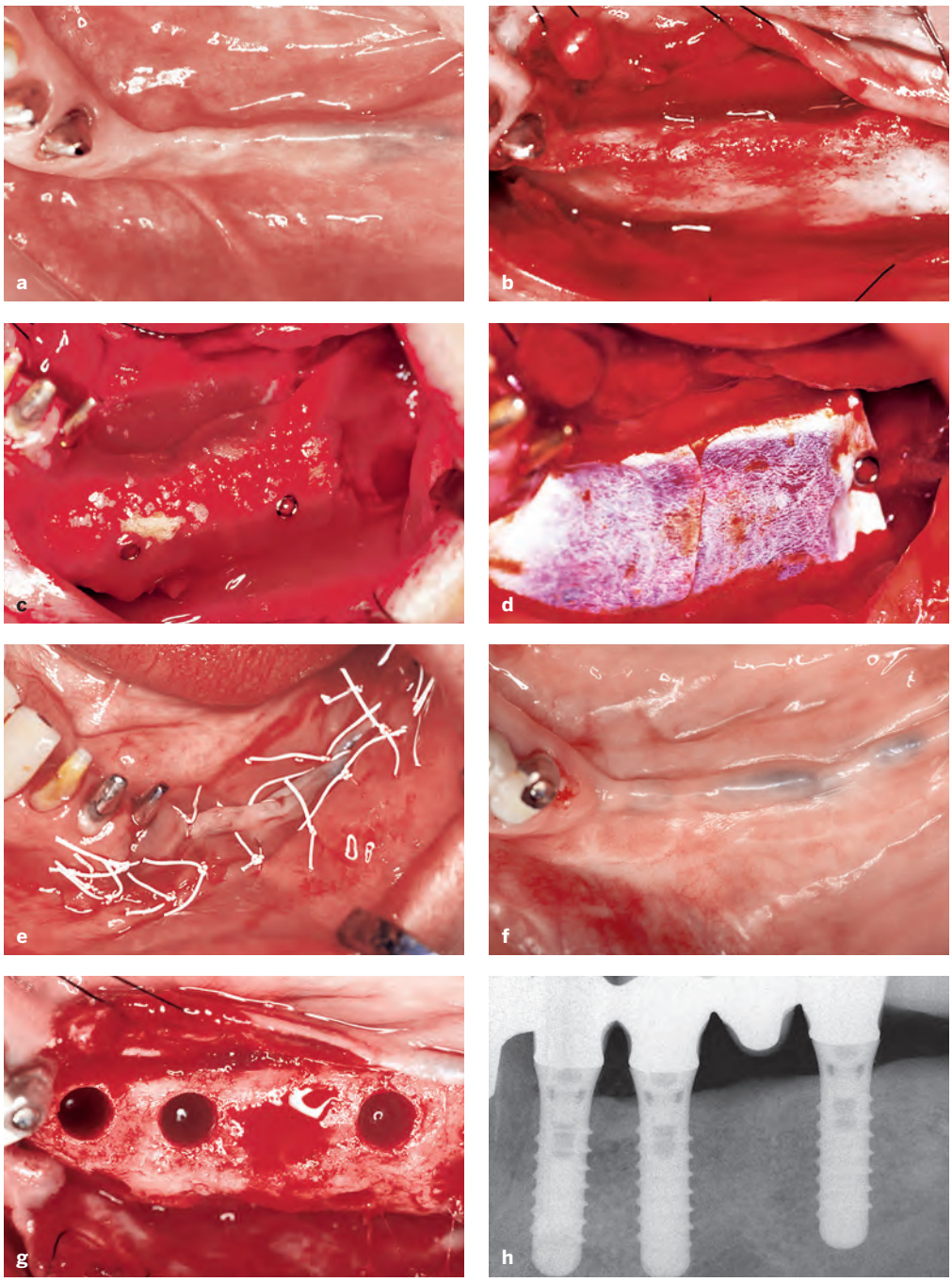

Fig 1-4 Case 3. *(a)* Preoperative view (1993). The occlusal view shows a distal extension situation in the left mandible. This woman's healed ridge was atrophic with a severe buccal flattening. *(b)* The intraoperative view shows a crest width of less than 3 mm. *(c)* Status following horizontal ridge augmentation with two block grafts harvested in the third molar area within the same flap. *(d)* The block grafts were covered with an ePTFE membrane. The membrane was stabilized with multiple miniscrews. *(e)* The surgery was completed with a tension-free wound closure with mattress and single sutures to achieve primary wound healing. *(f)* Clinical status after 6 months of healing free from complications. *(g)* Following flap elevation and membrane removal, an excellent augmentation outcome is visible in the areas of the first premolar and first molar, allowing for implants to be placed. *(h)* Following successful restoration, the periapical radiograph at the 1-year examination (1994) shows stable bone crest levels at all three tissue-level implants. →

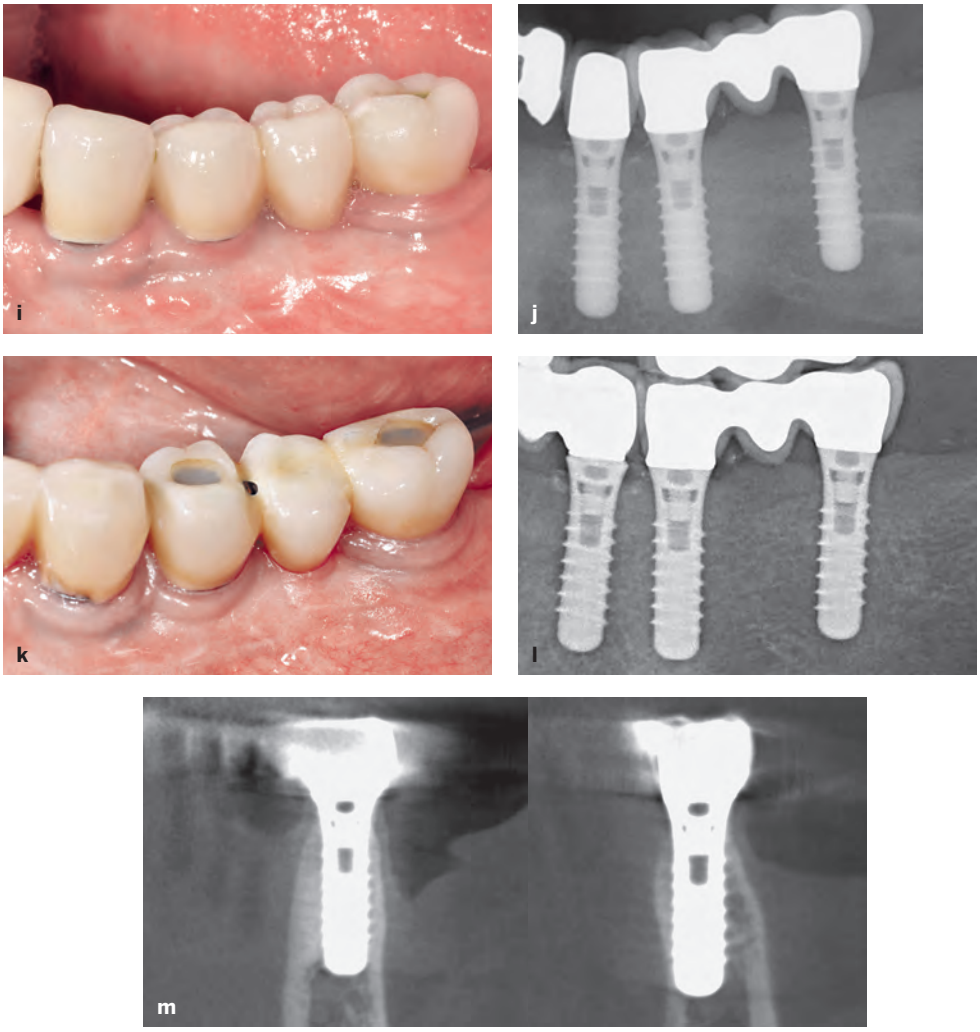

Fig 1-4 Case 3. *(cont) (i)* Clinical status at the 15-year examination. The peri-implant mucosa is stable but shows some signs of mucositis. *(j)* The radiograph confirms stable bone crest levels at all three tissue-level implants. *(k)* Clinical view at the 25-year follow-up examination (2019). The patient is now 85 years old, and the plaque control is no longer optimal. The mucosa around the tissue-level implants with a machined implant surface in the neck area shows very stable peri-implant tissues. *(l)* The periapical radiograph confirms stable bone crest levels at all three tissue-level implants after 25 years of function. *(m)* A CBCT scan is taken to examine the peri-implant bone volume. The orofacial cuts demonstrate fully intact buccal bone walls at the two implants in the first premolar and first molar sites, where a block graft augmentation with GBR was done in 1993.

Fig 1-5 Photo of the expert meeting in 1994 in Arizona with (from the left) Danny Buser, Bill Becker, Sascha Jovanovic, and Massimo Simion.

Box 1-1 Objectives for improvements of the GBR technique in the mid 1990s

- Improve the predictability of successful outcomes following GBR
- Reduce the rate of complications due to membrane exposure and membrane infections
- Make the GBR technique more user friendly, with easier application of the membrane during surgery
- Make GBR more patient friendly by eliminating a second surgical procedure for membrane removal whenever possible, and by reducing healing periods as much as possible

In 1994, an expert meeting took place in the United States to discuss the potential and the limitations of the GBR technique used in daily practice after 5 years of clinical experience (Fig 1-5). This meeting clearly showed that improvements of the GBR technique were needed to allow more widespread use in implant patients. The experts agreed that the GBR technique—based on the use of ePTFE membranes in combination with bone grafts or bone substitutes—had the following weaknesses and shortcomings:

- A significant rate of membrane exposures due to soft tissue dehiscences, often leading to local infection beneath the membrane and subsequently to a compromised regenerative outcome of the GBR procedure.[57–60]
- Difficult handling of the membrane during surgery due to its hydrophobic properties, requiring stabilization of the membrane with miniscrews or pins.[55,56,61]
- The need for a second surgical procedure to remove the bioinert, nonresorbable membrane, thereby increasing the morbidity and overall treatment time for the patient.

During this meeting, objectives were defined to improve the predictability and attractiveness of GBR procedures both for implant patients and for clinicians (Box 1-1).

It was clear to the participants at this expert meeting that these objectives could only be achieved with the use of a bioresorbable membrane. This trend was again initiated in the field of GTR, with the

introduction of the first bioresorbable membranes in the early 1990s.[62,63] Subsequently, numerous animal studies were performed to examine different bioresorbable membranes for GBR procedures.[64–74] In general, two different groups of bioresorbable membranes were evaluated[75]:

- Polymeric membranes made of polylactic or polyglycolic acid
- Collagen membranes produced from various animal sources

Paralleling these preclinical studies, clinicians started to use bioresorbable membranes in patients. The first published clinical reports predominantly tested collagen membranes,[76–80] and today, collagen membranes are routinely used in daily practice for GBR procedures.

In addition to selecting an appropriate barrier membrane, the selection of appropriate bone fillers for GBR procedures is just as important for the regenerative outcome of GBR procedures. In the early 1990s, autogenous bone chips were primarily used from a mechanical point of view. The role of these filler particles was to support the membrane to avoid a membrane collapse during healing. In the mid 1990s, a first preclinical study in minipigs by Buser et al[81] helped us to understand that bone fillers have different biologic characteristics in terms of their osteogenic potential and rate of filler substitution during bone remodeling.

The various biomaterials used for GBR procedures, such as bone grafts, bone substitutes, and barrier membranes, are also discussed in chapter 2.

Routine Application and Fine-Tuning Phase of GBR

Around the year 2000, GBR entered a phase of routine application in daily practice. Since then, the GBR technique has been the standard of care for the regeneration of localized bony defects in implant patients. This was confirmed in 2007 in a systematic review by Aghaloo and Moy,[82] who demonstrated that implants placed with the GBR procedure have favorable survival and success rates, and the GBR procedure was the only well-documented surgical technique among various surgical techniques used for localized ridge augmentation. The only other scientifically well-documented surgical technique for bone augmentation at that time was sinus grafting and sinus floor elevation in the posterior maxilla.

Over the past 20 years, however, significant progress has been made with GBR procedures, thanks to new developments in technology and a much better understanding of the tissue and graft biology involved.

The most important improvements are as follows:

- The development of a much better 3D radiographic technique based on CBCT
- Much greater knowledge of tissue biology in postextraction sites
- A much better understanding of the biologic characteristics of bone grafts and bone substitutes
- The development of new narrow-diameter implants

CBCT as the new 3D radiographic methodology

The development of the CBCT technique started in the late 1990s with a first publication by Mozzo et al,[83] and it represents probably one of the most important improvements in implant dentistry in the past 20 years. This new 3D radiographic technique allowed cross-sectional imaging with much better image quality and a clear reduction in radiation exposure when compared with the computed tomography (CT) technology used for dentistry in the 1990s. The CBCT technique allows cross-sectional imaging not only for the preoperative examination of patients, but also for the follow-up documentation of bone augmentation procedures.[84,85] During preoperative examination, CBCT helps to assess the extent of bone deficiencies in potential implant sites, and hence to categorize defect morphologies. These aspects are discussed in detail in chapter 5. In addition, CBCT is also one of the basic techniques necessary for the use of digital technology, including computer-assisted implant surgery (CAIS) in patients.

Improved knowledge of tissue biology in postextraction sites

The progress in this field was initiated around 2004 to 2005 by fundamental studies on bone alterations in postextraction sites performed by the group of Lindhe et al. In the beginning, a series of experimental studies in beagle dogs helped to explain the concept of bundle bone resorption postextraction.[86,87] These studies were followed by a number of clinical studies using the CBCT technique (for review, see Chappuis et al[88]). This new knowledge was fundamental for the definition of selection criteria used in postextraction implant placement. The current knowledge of hard and soft tissue alterations is discussed in detail in chapter 4, and the selection criteria for the different treatment options are presented in chapter 6.

Better understanding of the biologic characteristics of bone grafts and bone substitutes

As mentioned in a previous paragraph, autogenous bone chips had already been utilized with GBR procedures in the late 1980s, but they were used primarily as membrane support to avoid membrane collapse during healing. In the late 1990s, a first preclinical study by Buser et al[81] in minipigs showed that bone fillers have different biologic characteristics. Autogenous bone chips have excellent osteogenic potential, fostering new bone formation during early healing, and have a high substitution rate during bone remodeling. The alternative bone fillers tested were all associated with much slower bone formation during early healing, but one of them showed an interesting low substitution rate. Subsequently, a series of experimental studies with various bone fillers were conducted by Jensen et al,[89–91] confirming the superiority of autogenous bone chips with regard to osteogenic potential in comparison with all other bone fillers tested. In contrast, these studies showed that some bone fillers had very good volume stability with a low substitution rate, such as deproteinized bovine bone mineral (DBBM), a bovine bone filler. This new insight into the biologic properties of bone grafts and bone substitutes increasingly favored the use of two bone fillers as a so-called *composite graft*, which can be used either as a two-layer or a mixed composite graft (see chapter 2).

In the 2010s, the characteristics of autogenous bone chips were further examined in a series of in vitro studies using cell cultures. The studies showed that these bone chips instantly release growth factors (GFs) such as transforming growth factor β1 (TGF-β1) and bone morphogenetic protein 2 (BMP-2) into the surrounding blood, both potent GFs for osteogenesis.[92–95] With this release of GFs, the blood containing them is called *bone-conditioned medium* (BCM). BCM is then able to biologically activate bone fillers and barrier membranes for GBR procedures.[96,97] All these details are presented in this textbook in a completely new chapter 3.

Development of new narrow-diameter implants made of a Ti-Zr alloy

Narrow-diameter implants (NDIs) made of commercially pure titanium (CPTi) were already available in the mid 1990s, but they had limited clinical applications because NDIs showed an increased fracture rate in daily practice due to fatigue fractures.[98] To reduce the risk of fracture, splinting NDIs to other implants was recommended at that time.[8] Around 2010, a new titanium-zirconium (Ti-Zr) alloy called Roxolid (Straumann) was introduced to the market. This new implant material offered much greater strength when compared with CPTi.[99] The stronger implant material was able to reduce the risk of fracture, and hence widened the range of applications in daily practice. In the meantime, NDIs became well documented by clinical studies and systematic reviews.[100–103] In the most recent patient pool analysis, covering 3 years (2014 to 2016) at the University of Bern, the frequency of NDIs clearly increased, to roughly 25%.[21] This means their use has remarkably more than doubled in a 6-year period.[20]

The utilization of NDIs has two advantages in daily practice. First, it allows the clinician to use a standard implant placement protocol without a simultaneous GBR procedure in borderline situations with a crest width of around 6 mm. Second, in case of a local bone defect, it optimizes the defect morphology following implant placement and hence reduces the frequency

of staged approach augmentation procedures. The benefit for patients is obvious, because it reduces not only morbidity, but also costs. These details are discussed in chapter 5 of this textbook.

All these developments have enabled us to fine-tune the GBR technique in the past 20 years, and the details of these aspects are discussed in the clinical chapters of this book.

Summary

Over the years, significant progress has been made with GBR procedures in implant patients. GBR has not only become the standard of care for the regeneration of localized bone defects in the alveolar ridge of potential implant patients, but it has been an important contributing factor for the rapid expansion of implant therapy in the past 20 years, as well as contributing to significant progress in the field of esthetic implant dentistry.

The procedures recommended in various clinical situations are presented step-by-step in chapters 6 to 13. The reader of this textbook will quickly realize that the recommended surgical techniques are rather conservative, following basic rules of bone augmentation procedures. This offers the clinician the most predictable approach to achieving a successful treatment outcome with a low risk of complications, and thus the ability to become a successful implant surgeon who is able to satisfy the high expectations of today's patients.

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2

Bone Regeneration in Membrane-Protected Defects

Dieter D. Bosshardt, MSc, PhD | *Simon S. Jensen, DDS, Dr odont* |
Daniel Buser, DDS, Prof em Dr med dent

A sufficient amount of living bone is required for both the esthetic outcome and the long-term success of dental implant therapy. In about 50% of implant sites, however, there is a need for a procedure that predictably generates enough bone volume for the placement of a dental implant. There are several options for the enhancement of bone formation, including (*1*) osteoinduction by autogenous bone grafts or the addition of growth factors; (*2*) osteoconduction provided by autogenous bone grafts or bone substitutes that serve as a scaffold for new bone formation; (*3*) transfer of stem cells or progenitor cells that differentiate into osteoblasts; (*4*) distraction osteogenesis; and (*5*) GBR using barrier membranes. Regardless of the method used, there is always an underlying basic biologic mechanism of bone healing.

Bone demonstrates a unique potential for regeneration, which is probably best illustrated by fracture repair. Bone is able to heal fractures or local defects with regenerated tissue of equally high structural organization, without leaving a scar. The mechanism of this healing pattern is often considered as a recapitulation of embryonic osteogenesis and growth. Because bone has a unique spontaneous healing capacity, the trick in reconstructive surgery is to harness this great regenerative potential to enhance bone formation for clinical applications. Thus, adequate bone augmentation or treatment of any bone defect requires a profound understanding of bone development and morphogenesis at the cellular and molecular levels. This chapter summarizes the development, structure, function, and regeneration of bone and discusses the pros and cons of the various biomaterials used for GBR to provide the biologic rationale for selecting appropriate biomaterial combinations for successful bone augmentation around dental implants in the long term.

Development and Structure of Bone

Functions

Bone certainly represents a great achievement in the evolution of supporting tissues. However, it has many additional functions. These include (*1*) mechanical body support, motion, and locomotion; (*2*) support of teeth for biting and crushing of food;

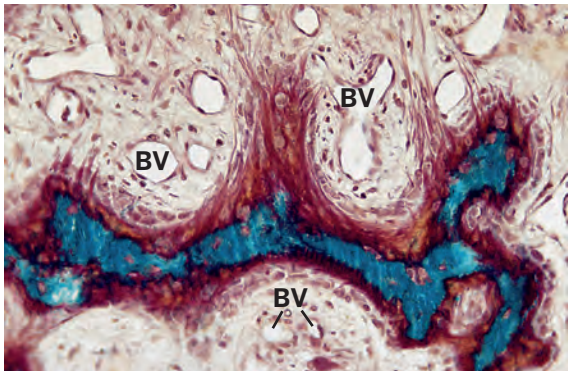


Fig 2-1 Light microscopic view of woven bone. This type of bone forms struts and ridges, which are always closely associated with blood vessels (BV; Goldner trichrome stain).

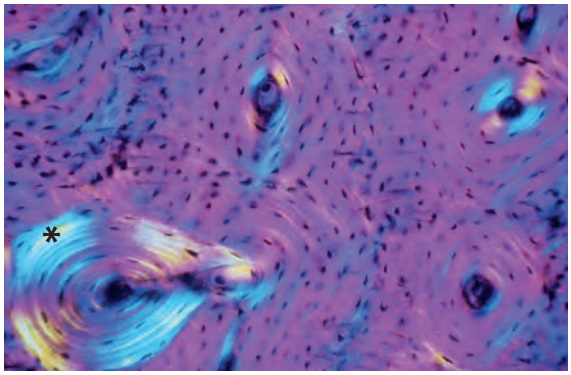


Fig 2-2 Primary and secondary osteons in equine cortical bone. In polarized light, secondary osteons *(asterisk)* reveal a clear lamellar pattern. The wall of primary osteons consists of primary parallel-fibered bone, which is less birefringent.

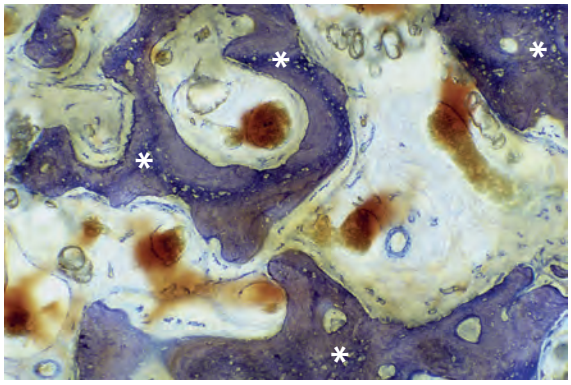


Fig 2-3 Light microscopic micrograph showing reinforcement of woven bone *(asterisks)* by parallel-fibered bone (toluidine blue surface stain).

(*3*) support and protection of the brain, spinal cord, and internal organs; (*4*) housing of bone marrow, which is the source of hematopoietic cells; and (*5*) calcium homeostasis. It is probably because these functions are of vital importance that bone possesses an exceptional capacity for self-healing, repair, and regeneration.

Bone types and structural organization

The mammalian skeleton consists of *long bones* and *flat bones*. Based on the orientation of the collagen fibrils, three types of bone tissue can be identified: woven bone, lamellar bone, and an intermediate type—the primary parallel-fibered bone.

Woven bone is formed predominantly in embryos and growing children and is replaced later by lamellar bone. In adults, woven bone reappears when accelerated bone formation is required, as in the bony callus during fracture repair and in pathologic conditions like Paget disease of bone, renal osteodystrophy, hyperparathyroidism, or fluorosis. In woven bone, the collagen fibrils are oriented in a random manner, and the interfibrillar spaces are comparatively wide.[1] Besides having intertwined collagen fibrils, woven bone is characterized by a high number of large osteocytes and a high mineral density (Fig 2-1).

Lamellar bone possesses a much more complex structure, characterized by matrix layers that consist of a parallel collagen fibril arrangement. One lamellar unit is about 3 to 5 µm wide, and the orientation of the fibrils changes from one lamella to another (Fig 2-2). Thus, lamellar bone may be regarded as a complex, plywood-like structure.[2]

Primary parallel-fibered bone is deposited in the early stages of bone formation, as well as during periosteal and endosteal bone apposition. Its collagen fibrils run parallel to the bone surface but lack lamellar

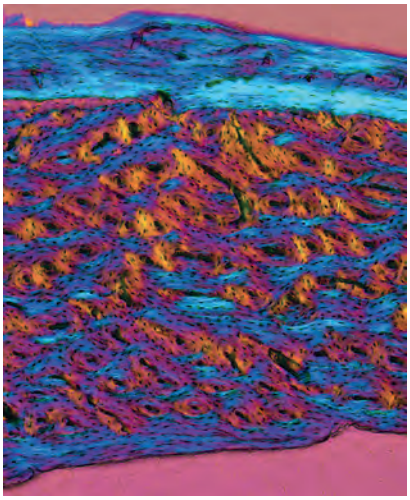


Fig 2-4 Polarized light micrograph of cortical bone from a rabbit tibia. Osteons built around Haversian canals are sandwiched between circumferential lamellae at the periosteal *(top)* and endosteal *(bottom)* surfaces.

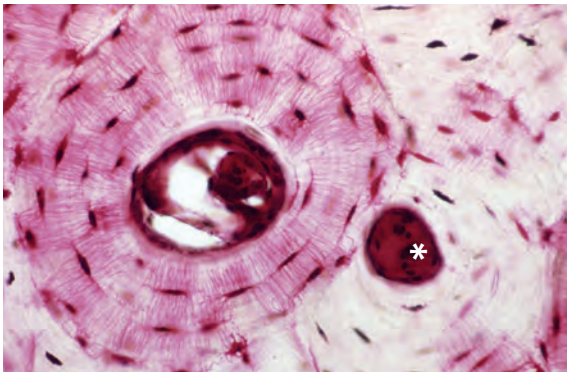


Fig 2-5 Osteons are metabolic units. Staining of osteocytes demonstrates the lacunar-canalicular system. Necrotic fragment of an osteon after obliteration of the Haversian canal *(asterisk;* undecalcified ground section; basic fuchsin stain).

organization (Fig 2-3). Primary parallel-fibered bone shares most physiologic properties with woven bone.

Mature bone consists of cortical (compact) and cancellous (trabecular or spongy) bone. Based on the orientation of the lamellae, cortical bone matrix is subdivided into different compartments. The basic structural units are the *osteons* or Haversian systems—longitudinally oriented cylindrical structures with vascular (Haversian) canals in the center. In secondary osteons, the wall consists of concentric lamellae, whereas primary osteons are characterized by a more primitive parallel-fibered bone matrix (see Fig 2-2). Along the periosteal and endosteal surfaces, appositional growth often results in packets of circumferential lamellae (Fig 2-4). Remnants of circumferential lamellae and of earlier generations of osteons occupy the remaining space in the form of interstitial lamellae. The osteocytes within these remnants of cortical remodeling activity are often cut off from their vascular supply and die[3] (Fig 2-5).

The trabeculae of cancellous bone are also composed of bone structural units, ie, packets or walls, separated or glued together by cement lines. They also reflect local remodeling in earlier periods of growth and cancellous bone turnover.[4]

Bone cells

Bone formation, maintenance, and repair are regulated by mesenchymal and bone-marrow-derived cells. Osteoblasts, osteocytes, and bone-lining cells are of mesenchymal origin, whereas osteoclasts belong to the monocyte/macrophage lineage and thus originate from bone marrow. Osteal macrophages are resident cells in bone tissue and have key functions in bone formation and remodeling.[5] Osteoblasts, bone-lining cells, and osteoclasts cover bone surfaces, whereas osteocytes are found in the interior of the bone matrix, and osteal macrophages are found in bone marrow cavities.

Osteoblasts are large cuboidal cells that form a single layer covering all periosteal or endosteal surfaces where bone formation is active.[6] They are polarized cells that secrete osteoid unidirectionally toward the bone surface. The nucleus of an osteoblast is ovoid, and its cytoplasm is filled with abundant rough endoplasmic reticulum and a prominent Golgi complex (Fig 2-6). Heterogeneity among osteoblasts seems to exist and may reflect differences between types of bone and/or anatomical sites.[6] The osteoblast is responsible for synthesis, assembly, and

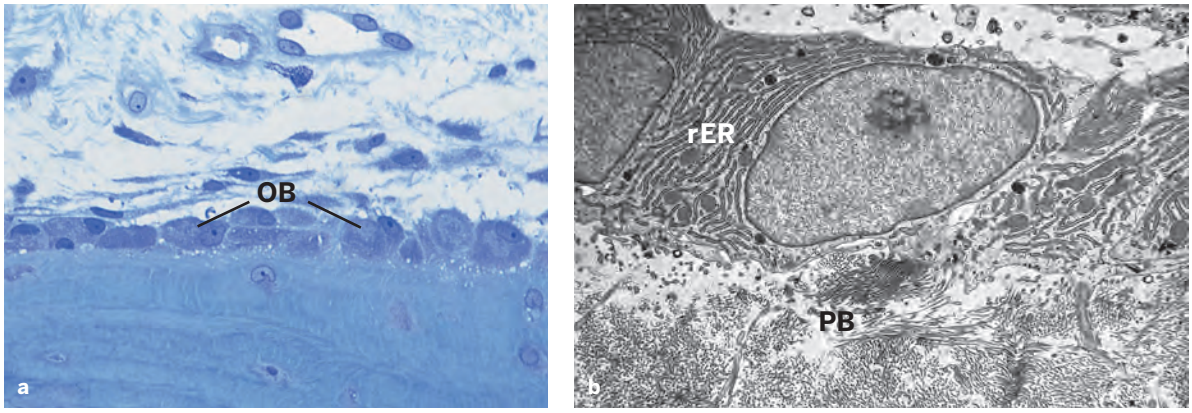


Fig 2-6 *(a)* Light micrograph showing a single layer of osteoblasts (OB) lining the bone matrix (toluidine blue stain). *(b)* Transmission electron micrograph illustrating osteoblasts with abundant rough endoplasmic reticulum (rER) lining the osteoid or prebone (PB).

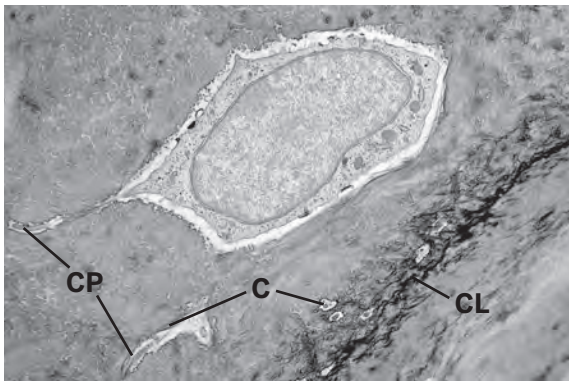


Fig 2-7 Transmission electron micrograph showing an osteocyte embedded in its lacuna next to a cement line (CL). Longitudinally and transversely cut canaliculi (C) containing cytoplasmic processes (CP) are visible close to the osteocyte.

mineralization of the bone matrix. Osteoblasts originate from mesenchymal stem cells in bone marrow.[7] Differentiation of cells of osteoblastic lineage is controlled by multiple transcription factors at various stages of their development. Runt-related transcription factor 2 (Runx2), also known as core-binding factor α1 (Cbfa1), and osterix (Osx), downstream from Runx2, are master switches for osteoblast differentiation.[8] The expression of Runx2 is not restricted to cells of the osteogenic and chondrogenic lineage,[9,10] and the expression of Runx2 in fully differentiated cells suggests additional roles in osteoblast function.

Some osteoblasts become osteocytes by inversion of matrix secretion or by entrapment through neighboring osteoblasts.[11] The speed of matrix deposition may determine the number of embedded osteocytes.[12] This is exemplified in woven bone, which is formed much more quickly than any other type of bone and possesses a high number of embedded osteocytes.[13] The osteocyte is trapped in the bone matrix in a lacuna (Fig 2-7), and neighboring osteocytes are interconnected by tiny cytoplasmic processes extending through a dense canalicular system. This lacunar-canalicular system allows for diffusion of nutrients, waste products, and signaling molecules for cell communication with neighboring osteocytes, osteoblasts, bone-lining cells, osteoclasts, and macrophages. It is indispensable for osteocyte survival because diffusion of nutrients and waste products through the heavily mineralized bone matrix is almost impossible. However, the transport capacity of this system also has limitations. In mammals, the critical transport distance to keep osteocytes alive is approximately 100 µm.[14] This explains why the wall thickness of both osteons and packets in trabecular bone rarely exceeds 100 µm. Healthy osteocytes are necessary for proper functioning of bone.[15] Osteocytes are more than passive cells buried in the bone matrix; they may

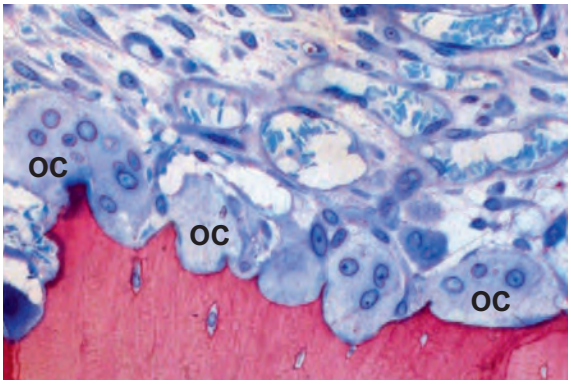
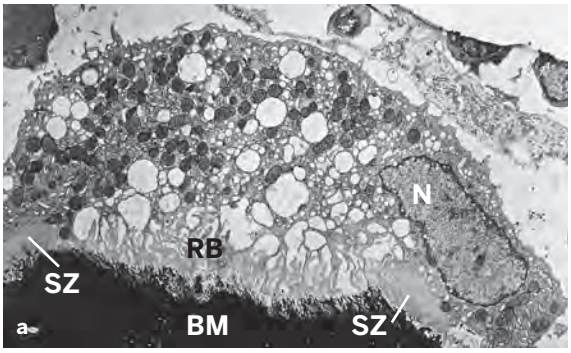


Fig 2-8 Light micrograph showing osteoclasts (OC) in Howship lacunae formed in the alveolar bone (fuchsin and toluidine blue stain).



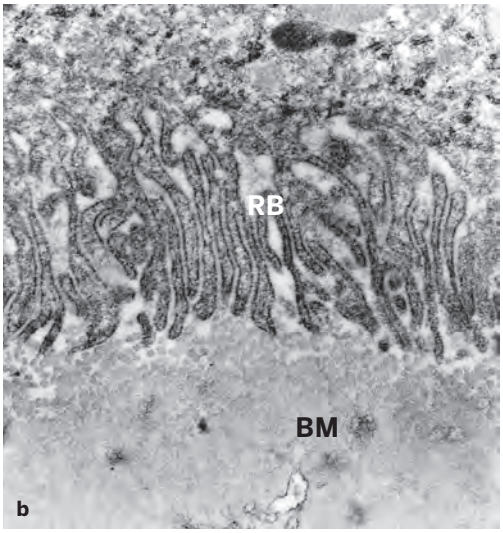


Fig 2-9 *(a)* Transmission electron micrograph showing an osteoclast with the ruffled border (RB), the sealing zone (SZ), numerous mitochondria, vesicles, and vacuoles, but only a single nuclear profile (N). The ruffled border represents the site of bone matrix (BM) dissolution and degradation (undecalcified ultrathin section). *(b)* Enlarged transmission electron micrograph of the ruffled border (RB) and bone matrix (BM; decalcified ultrathin section).

actively participate in bone homeostasis through their involvement in bone remodeling, ion exchange, and sensing of mechanical signals.[15,16] Importantly, osteocytes synthesize sclerostin, a negative regulator of bone formation,[17] and are the main source of receptor activator of nuclear factor-κB ligand (RANKL), which is required for osteoclast differentiation and activity.[18]

The bone-lining cell is the third cell type belonging to the osteoblast family. It is regarded as an inactive osteoblast covering the bone surface. Bone-lining cells are flat and have a reduced armamentarium of cytoplasmic organelles, which is indicative of low activity of both cell metabolism and protein synthesis. They are therefore also called *inactive* or *resting osteoblasts*. Bone-lining cells may participate in the initiation of resorption by release of osteoclast activation factors and by active contraction, which is thought to expose the bone surface for the attachment of osteoclasts.[19]

The osteoclast is a large, multinucleated cell. Osteoclasts differ morphologically from other giant cells, especially from foreign-body giant cells, and are conventionally identified by their location in a resorption cavity, the Howship lacuna (Fig 2-8). Their size varies from 30 to 100 µm, and the number of nuclei varies roughly from 3 to 30. Their primary function is to degrade bone matrix, a process called *bone resorption*. The cytoplasm is acidophilic and often contains vacuoles (Fig 2-9a). The marginal area of the osteoclast adheres to the mineralized surface and seals off the actual resorption chamber by the so-called *sealing zone* (clear zone; see Fig 2-9a). In the central part of this chamber, the cell surface is enlarged by numerous cytoplasmic undulations forming a ruffled border (Fig 2-9b). Through the enlarged cell membrane, hydrogen ions and proteolytic enzymes are released to dissolve the mineral crystals and to degrade the organic bone

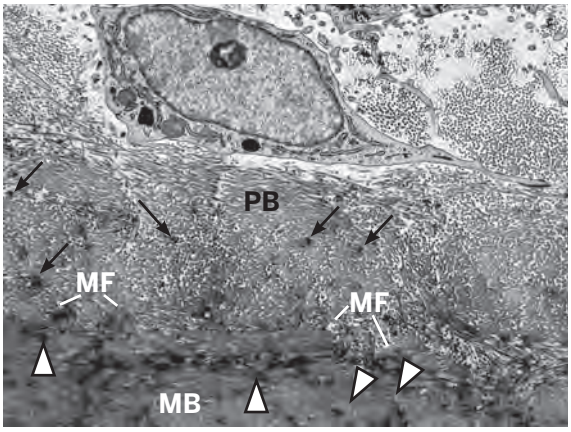


Fig 2-10 Transmission electron micrograph illustrating an osteoblast, the osteoid or prebone (PB), the mineralization front (MF), and the mineralized bone matrix (MB). Mineralization foci (*arrows*) and "gray patches" (*arrowheads*) are visible in the osteoid and in the mineralized bone matrix, respectively.

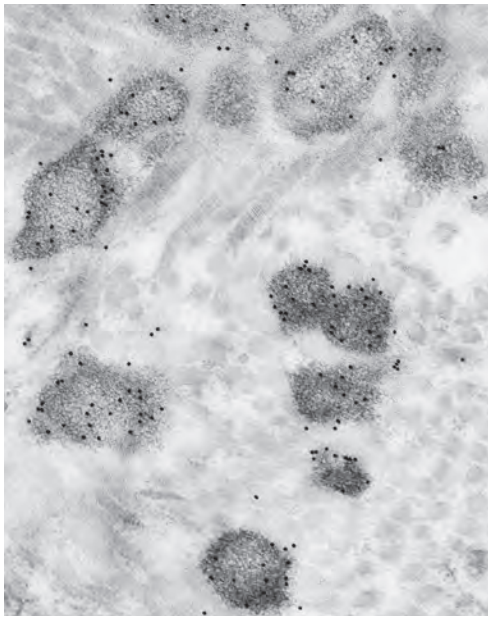


Fig 2-11 High-resolution immunocytochemistry showing an association between gold particle labeling for osteopontin and mineralization foci in the osteoid.

matrix. Osteoclasts originate from hematopoietic stem cells and develop from the self-fusion of macrophages.[20] Because of their origin, it is not surprising that many growth factors and transcription factors that are involved in hematopoietic differentiation of cells other than osteoclasts also affect osteoclast differentiation.[8] Marker proteins expressed by osteoclasts include tartrate-resistant acid phosphatase (TRAP),[21] cathepsin K, vitronectin, calcitonin, macrophage colony-stimulating factor (M-CSF), and receptor activator of nuclear factor-κB (RANK).

Macrophages in bone (osteal macrophages), another type of cells of hematopoietic origin, are also called *osteomacs*. These resident cells play substantial roles in bone biology, with key functions in regulating bone formation and remodeling. Furthermore, they are among the first cells that come in contact with implanted biomaterials used for GBR, and they can differentiate toward classic M1 or M2 macrophages or subsequently fuse into osteoclasts or other multinucleated giant cells.[5]

Bone matrix formation and mineralization

The osteoblast synthesizes a mixture of macromolecules that are secreted into the extracellular milieu to form the bone matrix—the osteoid or prebone—which consists of water, mineral, collagens, and noncollagenous macromolecules, the latter usually called *noncollagenous proteins*. The biochemical composition of bone has previously been reviewed,[22–27] and collagens play structural and morphogenic roles.[28] In mineralized tissues, they interact with various noncollagenous proteins and provide a scaffold for the accommodation of the mineral crystals.[29] The noncollagenous proteins of bone can roughly be classified into glycoproteins, proteoglycans, plasma-derived proteins, growth factors, and other macromolecules. In addition to its structural function, the bone matrix harbors molecules that play roles in biomineralization and matrix-cell interactions, and it also serves as a reservoir for growth factors and cytokines that may be released while osteoclasts resorb the bone matrix.

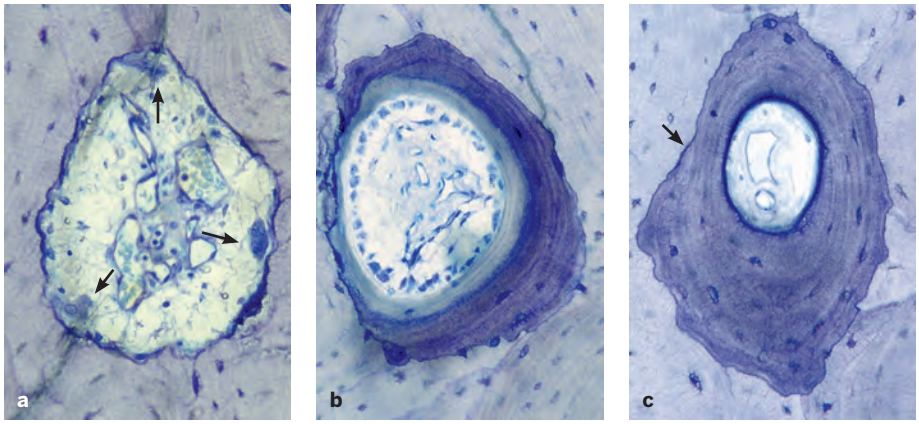


Fig 2-12 Cortical bone remodeling in the humerus of an adult man. *(a)* Resorption canal with osteoclasts *(arrows)*. *(b)* Concentric lamellae deposited by osteoblasts. *(c)* Completed secondary osteon, bound by a cement line *(arrow;* transverse sections through evolving secondary osteons; toluidine blue surface stain).

At a certain distance from the osteoblast, at the mineralization front, the osteoid converts into mineralized bone. The mineralization of woven bone is initiated by matrix vesicles. In contrast, matrix vesicles are rarely seen in the osteoid of mineralizing lamellar bone. However, the first mineral to appear among the collagen fibrils may be found at small discrete foci that are distributed within the osteoid and accumulate at the mineralization front (Fig 2-10). The colocalization of noncollagenous bone proteins such as osteopontin (Fig 2-11) and bone sialoprotein with these small mineralization foci and with amorphous gray or reticular patches of mineralized bone indicates the association of these proteins with the mineralization process. Bone acidic glycoprotein 75 and osteocalcin, on the other hand, show a diffuse distribution pattern throughout the mineralized bone matrix.[22]

Bone Modeling and Remodeling

Structural aspects

Throughout life, the skeleton undergoes continuous physiologic remodeling, which serves the purpose of repair and mechanical adaptation. The terms *modeling* and *remodeling* often cause confusion. Modeling indicates a change in shape, whereas remodeling refers to tissue replacement or substitution without a change in architecture. Cortical bone remodeling may be distinguished from trabecular bone remodeling.

Cortical bone

The fundamental structural unit of cortical bone remodeling is the osteon (see Fig 2-2). Primary osteons are formed during appositional growth, whereas secondary osteons are the result of matrix substitution. The remodeling sequence is illustrated in transverse sections in Fig 2-12. First, a resorption canal is formed by osteoclasts. Later, osteoblasts appear and start refilling the canal with concentric lamellae. In compact human bone, completed secondary osteons have an outer diameter of 200 to 250 µm, with the central vascular canal—the Haversian canal—measuring 50 to 80 µm.[30] As a coherent cylindrical structure, secondary osteons rarely measure more than 2 to 3 mm in length. At intervals of 0.5 to 1.0 mm, they are interconnected by transverse vascular channels called *Volkmann canals*. Longitudinal sections of newly formed osteons have shown that bone resorption and deposition are coupled in time and space and occur in discrete remodeling

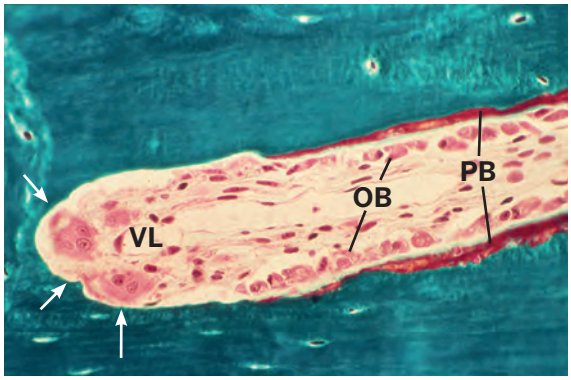


Fig 2-13 Longitudinal section through the tip of an evolving secondary osteon (bone metabolizing unit) during fracture repair in a canine radius. The *arrows* show the osteoclastic cutting cone. VL, vascular loop; OB, osteoblasts; PB, osteoid or prebone seam (microtome section, Goldner trichrome stain).

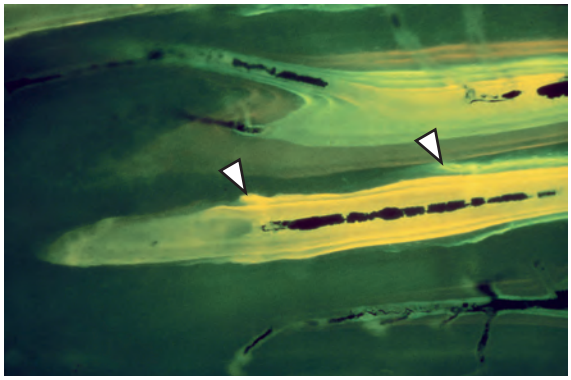


Fig 2-14 Sequential polychrome labeling of a bone metabolizing unit at weekly intervals *(arrowheads)* to measure the daily osteoclastic resorption rate.

sites called *bone metabolizing units* (BMUs). At the advancing front of a resorption canal, osteoclasts are assembled in a cutting cone (Fig 2-13). While the osteoclasts advance longitudinally, they widen the resorption canal up to its final diameter. The tip of a vessel loop follows immediately behind the osteoclasts. This loop lies in the center of the canal and is surrounded by perivascular cells thought to include osteoblast precursor cells. In the reversal phase (ie, between bone resorption and bone matrix deposition), the wall of the canal is lined by mononuclear cells. Further in the back of the cutting cone, osteoblasts appear and deposit the lamellar bone matrix, which will later mineralize. Depending on the species, completion of the osteon requires 2 to 4 months. These measurements and calculations are based on sequential fluorochrome labeling (Fig 2-14) and also allow an accurate determination of the resorption rate of the osteoclast in longitudinal sections, which amounts to 50 to 60 µm per day in dogs.[31–33]

Cancellous bone

While the original architecture of the trabecular framework is determined by the growth pattern, modeling in the spongiosa specifically changes the architecture of cancellous bone throughout life. The trabecular network undergoes profound changes that result in a structural adaptation to the prevailing functional load, or, as often stated, "according to Wolff's law."[34] This adaptation enables bone to withstand a given stress with a minimum amount of material. The mechanism of functional adaptation is not fully understood, and at present Wolff's law just offers a convenient way to accept it as a fact, without being forced to look for other explanations.

Bone remodeling improves the quality of the tissue with regard to both its mechanical and its metabolic properties. Remodeling of trabecular bone replaces discrete portions (or packets) with new lamellar bone (Fig 2-15). Formation of a new packet begins with local recruitment of osteoclasts that form a cavity on the trabecular surface. The mean depth of these cavities is around 50 µm, and it rarely exceeds 70 µm. At the end of this resorptive phase, and after a short intermission or reversal phase, osteoblasts start depositing new bone matrix during the formative phase. In analogy to the osteons, the new packet is considered a bone structural unit (BSU), and the cell populations involved in its formation are BMUs. Considering the extent of the trabecular surface in the human skeleton, the control and dynamics of cancellous bone remodeling play an important role in the pathogenesis of metabolic bone disorders, particularly in osteoporosis.

Cement lines

A cement line is a very characteristic structural entity of bone. It delineates and demarcates the interface between new and old bone. Two types of cement lines are distinguished: resting lines and reversal lines.

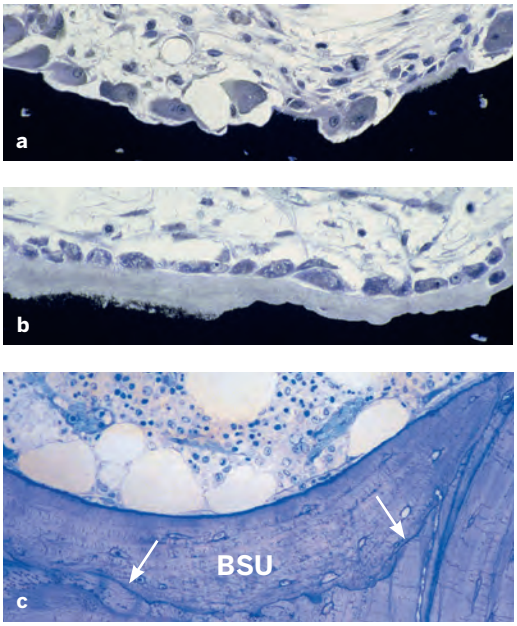


Fig 2-15 Trabecular bone remodeling in a human iliac crest biopsy. *(a)* Resorptive phase (Von Kossa-McNeal stain). *(b)* Early formative phase (Von Kossa-McNeal stain). *(c)* Newly formed packet (bone structural unit [BSU]), clearly delineated by a reversal or cement line *(arrows;* toluidine blue surface stain).

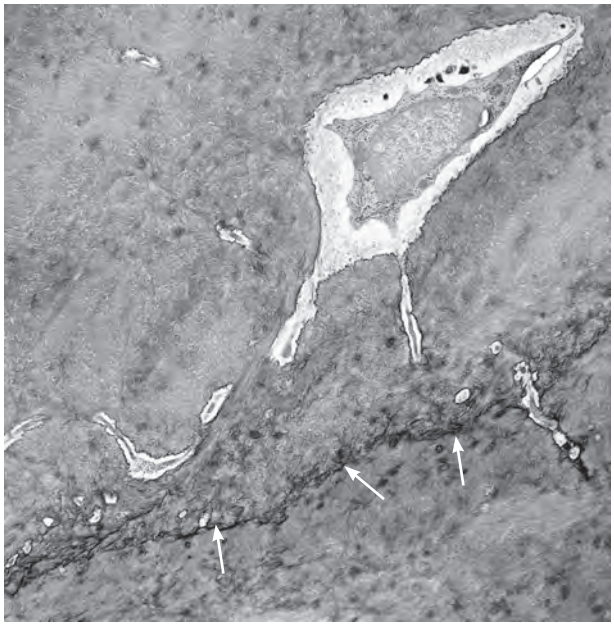


Fig 2-16 Transmission electron micrograph showing a cement line *(arrows)* at the interface between old and new bone.

Resting lines are smooth and strictly parallel to the lamellae. They are formed when bone formation is arrested and, after a resting period, resumes again. *Reversal lines* are formed during the reversal phase, ie, they constitute the matrix that is deposited directly against a bone surface previously resorbed by osteoclasts (Fig 2-16). Resting lines, which are produced by osteoblasts at sites not exposed to osteoclastic resorption, show structure and composition similar to reversal lines.

Secondary osteons are always separated from or connected to the surrounding older bone matrix by a cement line. Likewise, the new packet formed after the completion of trabecular bone remodeling is separated from the older bone matrix by a cement line. Howship lacunae left behind by the osteoclasts give the cement line a crenated appearance (see Figs 2-12c and 2-15c). The number of cement lines—both resting and reversal—indicates the intensity of matrix turnover.

Molecular aspects

Normal bone remodeling and thus bone mass maintenance depends on a delicate balance between bone formation and resorption involving a variety of cell types, including at least osteoclasts, osteoblasts, osteocytes, bone-lining cells, macrophages, and blood vessels, but also cells from the immune system, which communicate with each other via signaling molecules (ie, cytokines and growth factors). The consequences of an imbalance in the expression of signaling molecules are metabolic bone disorders or diseases like Paget disease of bone, osteopetrosis, osteoporosis, arthritis, or bone loss in periodontitis.[35,36] Regulation of bone remodeling is under both systemic and local control. Local factors are operative in a paracrine and autocrine fashion, and osteoblasts, osteoclasts, osteocytes, and inflammatory/immune cells function as both sources and targets of signaling molecules. Numerous cytokines and growth factors have anabolic and/or catabolic effects on bone formation.[37,38] Among

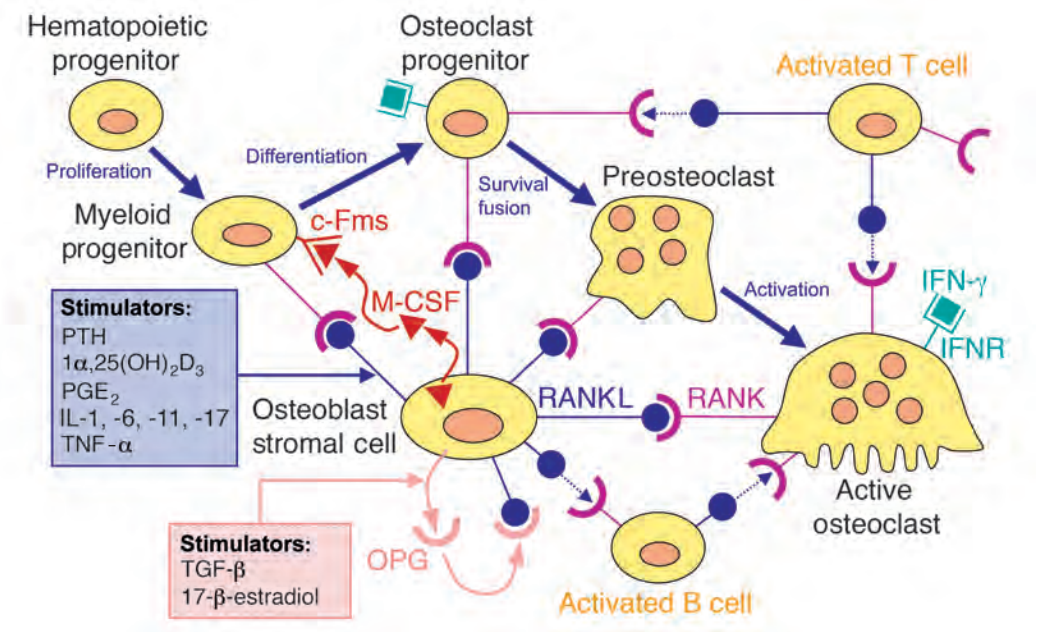


Fig 2-17 RANKL/RANK/OPG system. PTH, parathyroid hormone; $1\alpha,25[OH]_2D_3$, $1\alpha,25$-dihydroxyvitamin D_3; PGE_2, prostaglandin E_2; IL-1, interleukin 1; IL-6, interleukin 6; IL-11, interleukin 11; IL-17, interleukin 17; TNF-α, tumor necrosis factor α; M-CSF, macrophage colony-stimulating factor; c-Fms, M-CSF receptor; RANK, receptor activator of nuclear factor-κB; RANK-L, RANK ligand; IFN-γ, interferon γ; IFNR, IFN-γ receptor; TGF-β, transforming growth factor β; OPG, osteoprotegerin.

these bone-regulatory molecules are parathyroid hormone; parathyroid hormone-related peptide; calcitonin; calcitriol (the active form of vitamin D); prostaglandin E_2; growth hormone; thyroid hormone; sex steroids (estrogen and testosterone); leptin; statins; interferon γ; tumor necrosis factor α (TNF-α); transforming growth factor α (TGF-α); TGF-β; bone morphogenetic proteins (BMPs); fibroblast growth factor (FGF); insulin-like growth factor 1 (IGF-1); platelet-derived growth factor (PDGF); interleukins (IL) 1, 6, 11, and 17; and sclerostin.

A breakthrough in bone and immunology research was the detection of the RANKL/RANK/OPG system, which was first identified in the late 1990s as a pivotal regulator of bone remodeling. Bone resorption is regulated by this system, consisting of RANK and its ligand RANKL (which are members of the TNF ligand and receptor families) and osteoprotegerin (OPG). RANKL is expressed mainly by osteocytes, but also by bone marrow stromal cells, osteoblasts, and certain fibroblasts, whereas RANK is expressed by osteoclast precursors and mature osteoclasts. The binding of RANK to RANKL induces osteoclast differentiation and activity and regulates osteoclast survival. OPG, however, is produced by osteoblasts, bone marrow stromal cells, and other cell types and is a soluble decoy receptor for RANKL that competes for this binding. Thus, OPG is a natural inhibitor of osteoclast differentiation and activation.

Any interference with this system can shift the balance between bone apposition and resorption. The expression of M-CSF plays an essential role in this regulatory system. Furthermore, it has been shown that a number of proinflammatory cytokines and growth factors, in particular IL-1 and TNF-α, regulate the expression of RANKL and OPG (Fig 2-17). The immune system modifies the balance between bone formation and resorption in a complex process involving T and B lymphocytes, dendritic cells, and cytokines. By the expression of RANKL on B cells, T cells, and marrow stromal cells, and the expression of RANK on osteoclast precursors, mature osteoclasts, T lymphocytes, B lymphocytes, and dendritic cells, these cells can directly influence bone resorption.[39–41]

The discovery of this important regulatory system, which links bone biology with immune cell biology, has opened the possibility of new therapeutic strategies. Attempts using recombinant OPG were successful in preventing bone resorption and loss. However, production of significant antibody titers in a patient given OPG brought this development to an early end. Another way of blocking RANK signaling is through the use of denosumab, a fully human monoclonal antibody against RANKL. Prolia (Amgen) is used in patients with osteoporosis, whereas Xgeva (Amgen) is used for the treatment of multiple myeloma, bone metastases from solid tumors, bone giant cell tumors, and hypercalcemia of malignancy refractory to bisphosphonate therapy. Like bisphosphonates, Prolia and Xgeva can cause osteonecrosis of the jaw. These findings have led to the new term *medication-related osteonecrosis of the jaw* (MRONJ), which replaces the older term *bisphosphonate-related osteonecrosis of the jaw* (BRONJ). To help prevent MRONJ, clinicians have to ask their patients if they take antiresorptive medication. MRONJ has also been described as a complication of cancer therapies that target angiogenesis. However, this association is more controversial. Use of antiangiogenic agents as a risk factor for MRONJ among patients receiving osteoclast inhibitors for cancer is more clearly established.

Biology of Bone Regeneration

Physiologic versus reparative regeneration

Regeneration is commonly understood as replacement of vanishing or lost components in the body by tissues or organs of equally high structural organization so that structure and function are completely restored. Physiologic regeneration is distinguished from reparative regeneration. Many tissues or organ systems undergo *physiologic regeneration*, ie, continuous replacement of cells or tissue elements. Remodeling of cortical and trabecular bone also represents regeneration; both cells and extracellular matrix are replaced. *Reparative regeneration* takes place when tissues are lost because of injury or disease. Bone has the unique potential to completely restore its original architecture, but there are certain limitations. The reconstruction of the original level of tissue organization occurs sequentially and closely repeats the pattern of bone formation occurring during development and growth. Likewise, some basic conditions have to be fulfilled, such as ample blood supply and mechanical stability provided by a solid base.

Activation of bone regeneration

Any bone lesion (fracture, defect, insertion of an implant, interruption of blood supply) activates wound healing and local bone regeneration by the release and local production of growth factors and other signaling molecules, as discussed in chapter 3 of this textbook. Bone is one of the richest sources of growth factors and other signaling molecules. Osteoinduction in its classic concept implies initiation of heterotopic (ectopic) bone formation; that is, bone formation at sites where bone physiologically does not normally exist. The term *osteoinduction*, however, is just as frequently applied if ossification is activated in contact with existing bones; that is, in the case of orthotopic bone induction. To avoid confusion, the term *bone activation* is preferred for orthotopic bone formation.

In heterotopic bone formation, inducible osteoprogenitor cells are found far from bone. These mesenchymal cells are abundant in subcutaneous connective tissue, skeletal muscles, and the spleen and kidney capsule. Their response to inductive stimuli, such as BMPs, is more complex than in orthotopic bone formation, and in fact mimics endochondral bone formation.[42]

After subcutaneous implantation of BMPs in rats, proliferation of mesenchymal cells starts after 3 to 4 days. From days 5 to 8 onward, cartilage develops, and within 1 day it starts to mineralize. Vascular invasion and bony substitution of the calcifying cartilage follows from days 10 to 11 and onward. Intermediate cartilage differentiation seems to be mandatory if induction acts on inducible osteoprogenitor cells. Importantly, the response is always indirect bone formation.

In orthotopic bone formation, osteoprogenitor cells are found in tissues in direct proximity to bone, such as in bone marrow stroma, periosteum, endosteum, and intracortical canals. These cells respond to inductive signals with proliferation and differentiation directly into osteoblasts. Thus, in orthotopic bone

induction, the inducing agents act on determined osteoprogenitor cells, and the cellular response is direct bone formation. The lag phase is short—seldom longer than 1 to 3 days—and the newly formed bone is laid down on preexisting bone surfaces.

Repair of bone defects

The repair of experimental bone defects is a good model for the study of bone regeneration. In contrast to fractures, defects are less subject to mechanical factors and to obstructions of the blood supply. Johner[43] examined the healing of bore holes with diameters of 0.1 to 1.0 mm in the rabbit tibia. Bone formation within these holes starts within a couple of days, without preceding osteoclastic resorption, and reveals a clear-cut size dependency. Holes with a diameter in the range of osteons (0.2 mm) are concentrically filled with lamellar bone. In larger holes, a scaffold of woven bone is formed first, and then lamellar bone is deposited in the newly formed intertrabecular spaces, which have a corresponding diameter of 150 to 200 µm. As in appositional growth, the matrix deposition rate of lamellar bone is restricted to a couple of microns per day, whereas woven bone rapidly bridges larger defects. After 4 weeks, both the small and the larger defects are completely filled with compact bone.

There is, however, a threshold for this rapid bridging by woven bone. This threshold lies around 1 mm for bore holes in rabbit cortical bone.[43] Experimental studies on bony ingrowth into porous acetabular components in canine hip joint arthroplasty demonstrate similar results,[44] summarized in the often-quoted phrase *osteogenic jumping distance*. This term indicates that bone is not able to cross gaps wider than 1 mm in one single jump. In the case of implants, the situation becomes even more difficult because bridging of the defects starts from the bony side only. This does not mean that larger holes or gaps will stay open indefinitely, but filling takes longer, and there is no doubt that bore holes of 3 to 5 mm persist for several weeks, if not months, until repair is completed.

The bone healing around dental implants has been analyzed in relation to the distance between the implant surface and the surrounding bone,[45] in relation to the implant surface characteristics,[46,47] and in relation to implant materials and surface topographic and chemical modifications.[48]

Guided Bone Regeneration

As outlined previously, bone tissue exhibits a remarkable regenerative potential and perfectly restores its original structure and mechanical properties. However, this capacity has limitations and may even fail if certain conditions are not fulfilled. Factors that impede or even prevent bone repair include (*1*) failure of vascular supply; (*2*) mechanical instability; (*3*) oversized defects; and (*4*) competing tissues of high proliferative activity. However, several options, alone or in combination, are available to promote and to support bone formation, including the following:

- Osteoinduction by growth factors
- Osteoconduction by autogenous bone grafts or substitutes
- Transfer of stem cells or progenitor cells that differentiate into osteoblasts
- Distraction osteogenesis
- GBR using barrier membranes

GBR, usually in combination with a bone grafting material, is the most widely used method of augmenting bone in routine dental practice. The use of barrier membranes for bone regeneration was adapted from studies on periodontal regeneration[49,50] and started in the mid 1980s with preclinical experiments.[51–53]

The GBR principle

The key principle of GBR is to keep undesirable cells from nonosteogenic tissues from interfering with bone regeneration. To this end, a physical barrier membrane is placed between the region to be augmented with new bone and the adjacent soft tissue. Because bone is a relatively slow-growing tissue, both fibroblasts and epithelial cells have the opportunity to occupy available space more efficiently during wound healing and to build up a soft connective tissue much faster than bone is able to grow. If the occlusive barrier function lasts long enough and if the barrier membrane is not exposed to the oral cavity, optimal conditions exist for the ingrowth of blood vessels from the resident bone, allowing stem cells and osteoprogenitor cells to differentiate into osteoblasts, which produce the bone matrix. In other words, the barrier membrane

creates a secluded space that allows bone to use its great, natural healing capacity in an undisturbed or protected manner.

It is noteworthy that the theory of total cell occlusion has been challenged by the awareness that nutrient transfer across the membrane may be important for successful bone regeneration.[54,55] Indeed, macroporous membranes were found to be more predictable for GBR and for guided tissue regeneration (GTR) procedures and more conducive to uncomplicated clinical management than occlusive membranes.[56,57] Very recently, the function of a membrane as a mere tissue-separating barrier has been further questioned. There is some evidence suggesting that certain membranes have functions beyond the barrier role.[58] These emerging data suggest that the membrane contributes actively to the regeneration of underlying bone defects. It would not be surprising if the specific macrophage response to a biomaterial is involved in this contributing effect.

Biomaterials used for GBR

Barrier membranes

In practice, the barrier membrane is placed in direct contact with the outer surface of the bone surrounding the defect, and the mucoperiosteal flap is then repositioned and sutured. Clinicians have access to a wide range of membrane materials for GBR. To select the material best suited for a specific clinical application, it is necessary to understand the basic requirements for membrane materials used in these indications. These basic characteristics include the following:

- Biocompatibility
- Cell occlusion (occlusivity, occlusiveness)
- Space-making and space-maintaining ability
- Tissue integration
- Degradability
- Clinical handling
- Susceptibility to complications

While in the pioneering phase of GBR in the late 1980s and 1990s, the nonresorbable expanded polytetrafluoroethylene (ePTFE) membrane prevailed, resorbable membranes were evaluated later. Both nonresorbable and bioresorbable membranes have inherent advantages and disadvantages.

Nonresorbable membranes

The most widely used nonresorbable membrane type is made of PTFE. It was originally developed in the late 1960s, and marketing began in 1971. PTFE is hydrophobic in nature and biologically inert, which makes this biomaterial nonresorbable. Thus, the major advantage is its excellent barrier function, whereas the major disadvantage is the need for a second surgery to remove the membrane. The ePTFE membrane is produced by exposing PTFE to high tensile strain, resulting in expansion and formation of a porous microstructure. The most commonly used ePTFE membrane has a two-part design with a dense portion and a less dense portion. The inner (central) dense portion, located over the defect space, has a pore size of less than 8 µm to allow fluid exchange while preventing infiltration of cells (Fig 2-18a). In contrast, the microstructure of the outer portion, which is in contact with the bone at the defect margin, is less dense; it has pores 20 to 25 µm wide and a surface structure that encourages blood clot adhesion and soft connective tissue attachment to and invasion into the membrane, ultimately leading to tissue integration (Fig 2-18b). In dentistry, ePTFE membranes became the standard for GTR and GBR procedures during the development phase of both techniques in the 1980s and early 1990s.[59]

High-density PTFE (dPTFE) is different from ePTFE in that it possesses pores in the submicron scale (0.2 µm). Bacterial adhesion and infiltration is eliminated because of its high density and small pore size. Primary soft tissue closure is not required, allowing tissue healing with a barrier membrane being exposed to the oral cavity.

To overcome the problem of membrane collapse, the PTFE membrane can be mechanically stabilized with titanium, which is known as a *titanium-reinforced PTFE*. Alternatively, membranes made of metal meshes consisting of titanium or titanium alloy and cobalt-chromium alloy may be used.[60]

As already mentioned, one big disadvantage of nonresorbable membranes is the need for their removal with an additional surgical intervention. Furthermore, complications associated with ePTFE membranes include difficult handling because of their hydrophobic nature, membrane collapse, and membrane exposure, often leading to infection and compromised regenerative outcome. These

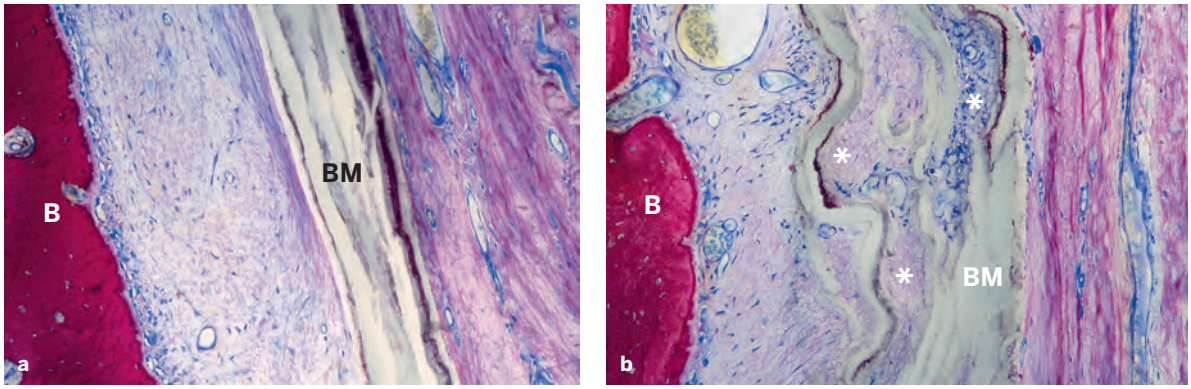


Fig 2-18 Soft and hard tissue compartments adjacent to an ePTFE barrier membrane (BM; ground sections; toluidine blue and basic fuchsin surface stain). *(a)* The dense portion of the barrier membrane separates an outer, mucosal compartment from an inner, soft connective tissue compartment that is mainly accessible from the bone (B) compartment. There are no signs of a foreign-body reaction. *(b)* The porous part of the barrier membrane *(asterisks)* allows ingrowth of blood vessels and cells into the gaps in the membrane.

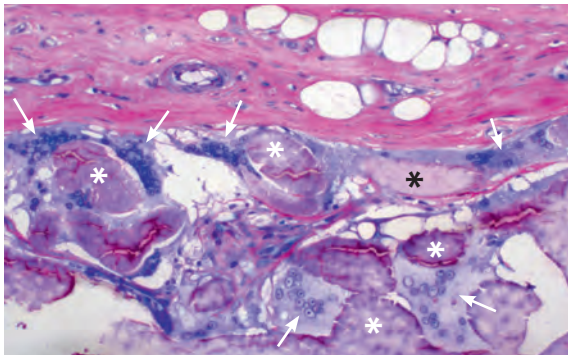


Fig 2-19 GLTC membrane at 12 weeks. Numerous large multinucleated giant cells *(arrows)* are interposed between an amorphous matrix *(asterisks)* that partly replaces the membrane material (undecalcified ground section; toluidine blue and basic fuchsin surface stain).

complications were the stimulus for the testing of another generation of biomaterials, the bioresorbable membranes.

Bioresorbable membranes

As the name indicates, bioresorbable or biodegradable membranes are broken down in the body and disappear over time. Thus, they have the advantage of eliminating the need for an additional surgery to remove the biomaterial. Two main categories of bioresorbable membranes exist: (*1*) synthetic polymers, and (*2*) polymers derived from various animal sources. Each membrane has distinct physicochemical properties and biologic effects. The degradation process has an important influence on the outcome, because (*1*) if degradation occurs too quickly, the biomaterial may not have a chance to fulfill its function as a barrier, and (*2*) products of degradation may contribute to unfavorable tissue responses, including foreign-body reactions, which may prevent tissue integration and bone formation and even result in bone resorption.[61] Most clinically used bioresorbable membranes are made of processed collagen or aliphatic polyesters.

Synthetic membranes

Synthetic polyesters used as barrier membrane biomaterials are polyglycolides (PGAs), polylactides (PLAs), or copolymers thereof. Other aliphatic polyesters used are polydioxanones[62] and trimethylene carbonates.[63] These synthetic biomaterials have both advantages and disadvantages. They can be made available in almost unlimited quantities. Another advantage is the ability of PGA, PLA, and their copolymers to completely biodegrade to carbon dioxide and water via the Krebs cycle.[64] Numerous factors are known to affect the degradation of bioresorbable polymers, such as their structure and chemical composition, molecular weight, shape, processing

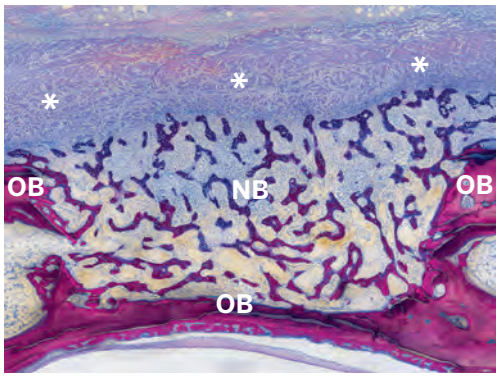


Fig 2-20 Light micrograph illustrating a non-cross-linked collagen membrane (Bio-Gide, Geistlich; *asterisks)* over a bone defect 2 weeks after its creation in the rabbit calvaria. New bone (NB) has loosely filled the defect space and has even surpassed the height of the pristine old bone (OB; undecalcified ground section; toluidine blue and basic fuchsin stain).

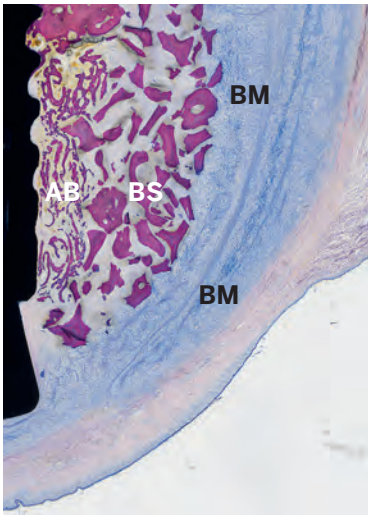


Fig 2-21 Light micrograph illustrating a buccal peri-implant bone defect 3 weeks after augmentation with autogenous bone chips (AB), a bone substitute (BS; Bio-Oss, Geistlich), and a non-cross-linked barrier membrane (BM; Bio-Gide). The double-layered barrier membrane perfectly covers the augmented region and blocks off from the oral mucosa (undecalcified ground section; toluidine blue and basic fuchsin stain).

conditions, sterilizing processes, physicochemical factors, and mechanism of hydrolysis.[65,66] The use of these polymers as bone plates, screws, and delivery vehicles for drugs and growth factors has been associated with inflammatory and foreign-body reactions in orthopedic and maxillofacial surgery and implant dentistry (Fig 2-19). In certain cases, even surgical debridement and removal of the biomaterial may be required.[67–71]

Collagen membranes

Most natural resorbable membranes are made of collagen from animal tissues, although human sources exist as well. The collagen membranes have different tissue sources, including bovine tendon, bovine dermis, calf skin, porcine dermis, and human skin from cadavers.[72] Collagen is the most abundant extracellular matrix protein in the body. Collagen has important structural functions, and it also supports cell attachment, cell differentiation, tissue repair, and tissue regeneration.[73,74] Collagen-based biomaterials are widely used in biomedical applications because they closely mimic the extracellular environment. Furthermore, collagen has low immunogenicity and is hemostatic.[75]

On the other hand, collagen membranes have unfavorable mechanical properties[76] and may provide an inadequate barrier function because they biodegrade too quickly through enzymatic activities of macrophages and polymorphonuclear neutrophils.[77–79] Biodegradation of collagen membranes into carbon dioxide and water is caused by endogenous collagenases.[75] To prolong the barrier function, a number of cross-linking technologies, such as ultraviolet light, formaldehyde, glutaraldehyde, diphenylphosphoryl azide, and hexamethylene diisocyanate, have been used.[80,81] The glutaraldehyde technique was reported to leave cytotoxic residues.[82] The degree of collagen cross-linking inversely affects the degradation rate.[83] Membrane ossification is a phenomenon observed in certain cross-linked collagen membranes. The modified collagen seems to trigger membrane ossification.[84,85]

Non-cross-linked collagen membranes are currently the membrane of choice for most GBR procedures, whereas nonresorbable membranes made of dPTFE may be recommended for selected vertical augmentation procedures only. Figures 2-20 and 2-21 show two excellent regenerative outcomes

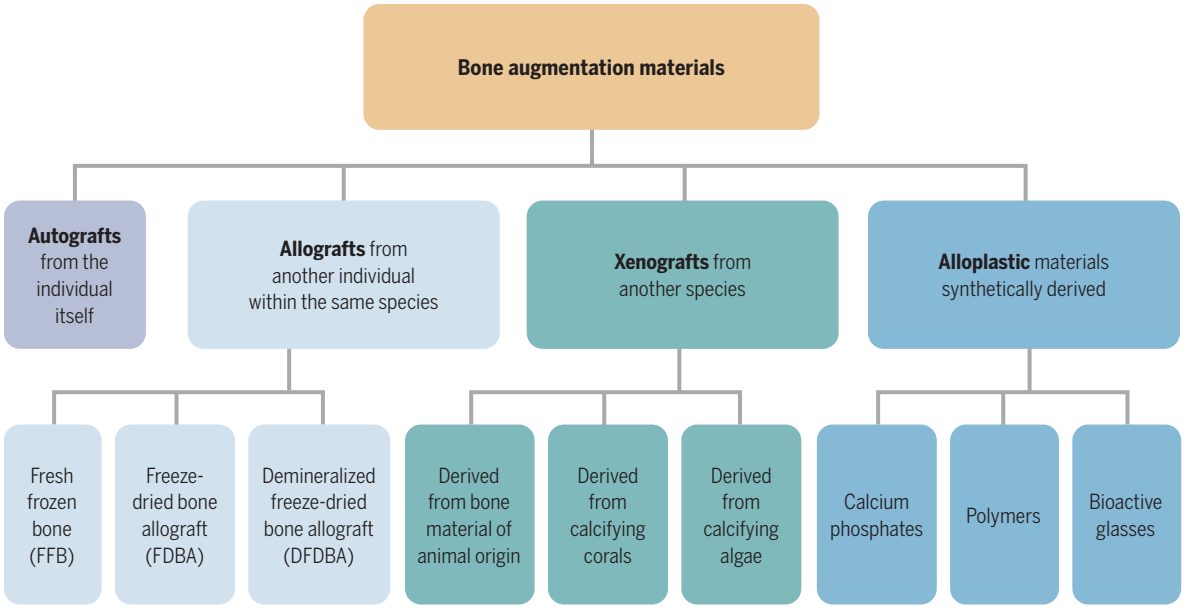


Fig 2-22 Classification of bone augmentation materials.

when a non-cross-linked collagen membrane was used to cover a bone defect either created in the rabbit calvaria or lateral to a dental implant, respectively.

Bone grafts and bone substitute materials

Because most bioresorbable membranes, and to a lesser degree ePTFE membranes, are largely incapable of maintaining defect space due to lack of sufficient rigidity, these membranes are often used in combination with autogenous bone grafts, bone substitute materials, or composite grafts. Bone fillers, however, serve many more purposes, including the following:

- Provide mechanical support to prevent membrane collapse
- Stabilize the blood clot
- Allow ingrowth of blood vessels
- Act as an osteoconductive scaffold for bone ingrowth
- May be osteoinductive
- May contain bone cells
- Become integrated in or replaced by bone
- Protect the augmented volume from resorption

The clinical indications for using bone filler materials range from grafting minor peri-implant bone defects to the regeneration of large continuity defects. Considering this wide range of purposes, it is to be expected that one single material cannot fulfill all requirements. Therefore, it will often be necessary to combine two or more materials to obtain a successful and predictable treatment outcome.

Bone filler materials can either be derived from the person being treated (autogenous bone grafts or autografts) or from an external source (bone substitute materials). The bone substitute materials are subdivided into allogeneic bone, xenogeneic bone, and alloplastic (synthetic) bone graft substitutes. Figure 2-22 shows a common classification of bone grafting materials. These materials possess different physical, chemical, and biologic characteristics. The biologic properties are commonly described as the following:

- Osteoconductive
- Osteoinductive
- Osteogenic

Osteoconductive materials possess a matrix that serves as a scaffold or framework. This matrix is used as a template and an enlarged solid base for bone deposition. Materials with *osteoinductive* properties contain proteins (BMPs) that stimulate and support proliferation and differentiation of uncommitted stem cells to become osteoblasts. *Osteogenic* means that the autogenous bone contains bone cells (bone-lining cells, osteoblasts, osteoblast uncommitted stem cells, and/or osteocytes) that are capable of directly or indirectly supporting bone formation at the transplantation site.

Autogenous bone grafts

Autogenous bone is a preferred bone graft material because it possesses osteoinductive, osteogenic, and osteoconductive properties. However, the harvesting of autogenous bone may require an additional surgical intervention, which increases the operative time, costs, intraoperative blood loss, pain, and recovery time. Moreover, it is associated with an increased risk of donor site morbidity (eg, increased postoperative pain, nerve injury, blood vessel injury, hematoma, infection, hernia formation, and cosmetic disadvantages). Finally, the supply of autogenous bone for grafting may be limited.

With transplantation of autogenous bone, bone-stimulating growth factors and viable osteogenic cells are brought to the recipient site.[86] The number of cells and the concentration of growth factors demonstrate great inter- and intraindividual variation and depend largely on patient's age, presence of systemic diseases, and location of the donor site. The growth factors comprise BMPs, TGF-β, IGF, PDGF, and FGF. They are mainly present in the bone matrix and are released either passively or during resorption of the autografts. The higher the absolute surface area of the autograft, the faster the growth factors are set free. This means that blocks of cancellous bone release growth factors more readily than blocks of compact bone and that particulate autografts demonstrate faster release of bone-stimulating growth factors than do blocks.[87] More about growth factor release from autogenous bone grafts is discussed in chapter 3.

The cells of primary interest in GBR procedures are the osteogenic cells (ie, osteoblasts, bone-lining cells, preosteoblasts, and pluripotent stem cells). These cells are most numerous in trabecular bone and least numerous in compact bone. The osteogenic potential is greater in young healthy individuals than in elderly patients. This is mainly because of a decreased proliferative capacity of osteoprogenitor cells in older individuals, rather than due to compromised function of the osteoblasts.[88] The presence of living osteocytes in transplanted autogenous bone confirms that cells can survive the transplantation procedure, but whether transplanted osteocytes have a function in bone formation is as little known as what this function may be. As discussed previously, osteocytes play a very important role in bone physiology.

Autografts can be harvested at different intraoral or extraoral locations (Table 2-1) and can be used in different forms (Table 2-2).[89] Both autografts and bone substitute materials can be used as block grafts, which are beyond the scope of this chapter, or as particulate grafts.

Autogenous particulate grafts

Bone particles are usually applied in an area where there is no need for mechanical strength, such as in peri-implant bone defects or in sinus floor elevation procedures. Particulate bone can be harvested either directly with a bone scraper (bone chips), with a piezoelectric device, with a bone collector (bone slurry or dust), or indirectly from a harvested bone block that is ground in a bone mill. The higher the relative surface area, the greater the osteoconductive and osteoinductive potential of autogenous bone. This is because the greater surface area allows more passive release of growth factors and more active liberation of growth factors by osteoclasts while they resorb the bone matrix.[87] While the osteopromotive potential of an autograft increases when it is particulated, the total number of intact osteogenic cells decreases through mechanical graft manipulation.[90] The smaller the particles, the more the stability of the graft decreases. In addition, the resorption rate increases considerably.[91] In a recent preclinical study, bone chips, bone slurries, bone harvested with a piezoelectric device, and bone ground by a bone mill were tested for their ability to support bone healing in defects created in the mandible of minipigs and covered with an ePTFE membrane.[92] The results showed that all bone defects

Table 2-1 Donor sites for autografts

	Region	Geometry	Volume	Resorption
Intraoral	Chin	Corticocancellous*	++	++
	Mandibular body/ramus	Cortical	++	+
	Nasal spine	Corticocancellous*	+	+++
	Maxillary tuberosity	Corticocancellous†	+	+++
	Zygomatic body	Corticocancellous†	+	+++
Extraoral	Illiac crest anterior/posterior	Corticocancellous†	+++	++
	Tibial condyle	Cancellous	++	+++
	Calvaria	Cortical	++	+
	Fibula (vascularized)	Corticocancellous†	+++	+

*More cortical than cancellous bone.
†More cancellous than cortical bone.
+, Sufficient bone for augmenting a single-tooth gap; ++, sufficient bone for up to two sinus augmentations; +++, sufficient bone for major inlay and onlay augmentations and reconstruction of continuity defects.

Table 2-2 Characteristics of autografts and their indications

Graft type	Osteogenic cells	Growth factors	Mechanical stability	Resorption	Indications
Cortical block	Moderate	Abundant	Pronounced	Minimal	Lateral and vertical ridge augmentation in a staged approach
Corticocancellous block	Abundant	Many	Substantial	Limited	Lateral and vertical ridge augmentation in a staged approach
Cortical particles	Few	Many	Moderate	Moderate	Minor lateral augmentations, peri-implant defects, fenestrations, and dehiscence-type defects simultaneous with implant placement Sinus augmentation procedures Packing around blocks Mixing with bone substitute materials
Cancellous particles	Many	Moderate	Limited	Substantial	Minor lateral augmentations, peri-implant defects, fenestrations, and dehiscence-type defects simultaneous with implant placement Sinus augmentation procedures Packing around blocks Mixing with bone substitute materials
Bone from bone scraper	Few	Moderate	Limited	Substantial	Minor lateral augmentations, peri-implant defects, fenestrations, and dehiscence-type defects simultaneous with implant placement Minor sinus augmentation procedures Mixing with bone substitute materials
Bone from bone collector	Few	Few	Minimal	Pronounced	Mixing with bone substitute materials

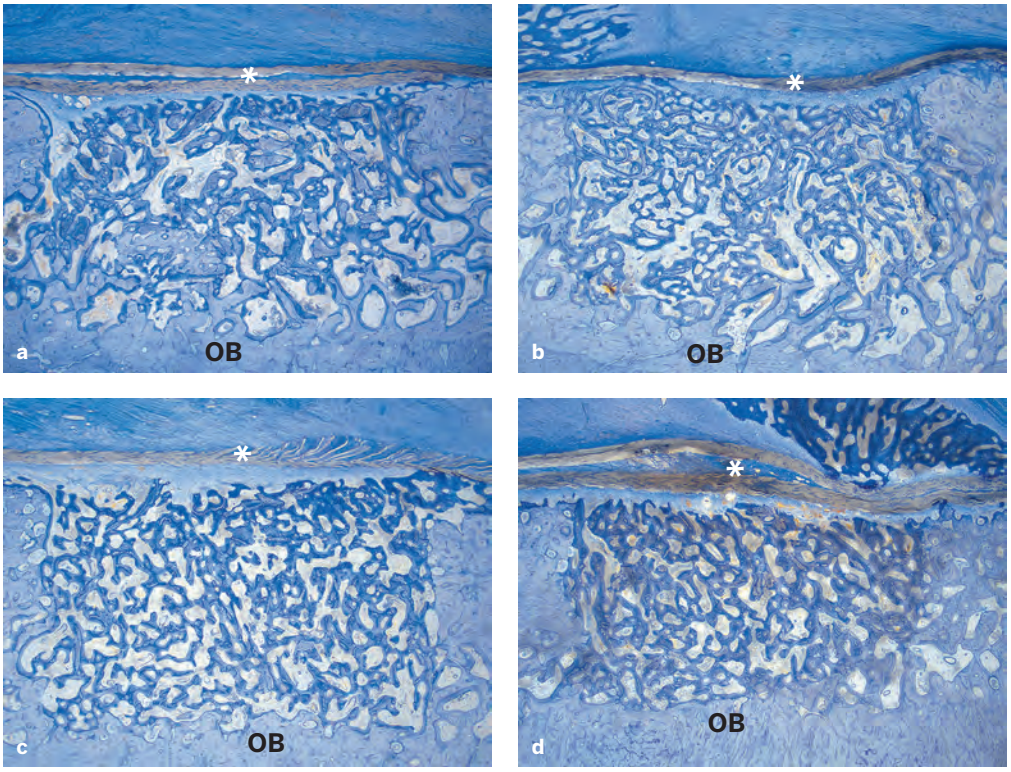


Fig 2-23 Histologic sections illustrating standardized bone defects in the minipig mandible (undecalcified ground sections; toluidine blue stain). Old bone (OB) and an ePTFE membrane *(asterisks)* delineate the defects. The defects were filled with autogenous bone. *(a)* Particulate from corticocancellous bone with a bone mill. *(b)* Bone chips harvested with a bone scraper. *(c)* Bone particles harvested with a piezoelectric instrument. *(d)* Bone slurry from a bone trap filter. After 4 weeks of healing, all types of autogenous bone grafts are embedded in a trabecular network of new bone that completely fills the defect areas.

healed with the same amount of new bone, regardless of the harvesting procedure and of the healing period (Fig 2-23). The highest number of osteoclasts was found after 1 to 2 weeks on the bone slurries, confirming that smaller particles trigger increased resorption (Fig 2-24).

A vast body of scientific evidence from experimental and clinical studies documents the suitability of particulate autografts for bone augmentation procedures when a highly osteogenic graft is needed. Nevertheless, the advantages and disadvantages of the different harvesting procedures in the context of clinical applications need to be discussed.

Autogenous bone from bone collectors. With this harvesting method, bone dust is collected through a filter that is connected to the suction device used during preparation of the implant bed. The philosophy is intriguing, because the acquisition of the autogenous bone graft causes no additional discomfort for the patient, but several disadvantages exist. Viable cells are clearly present in lower numbers than in other autografts,[90,93] the amount of bone that can be collected can only cover small peri-implant defects, and contamination of the graft with bacteria from the oral cavity occurs frequently.[94]

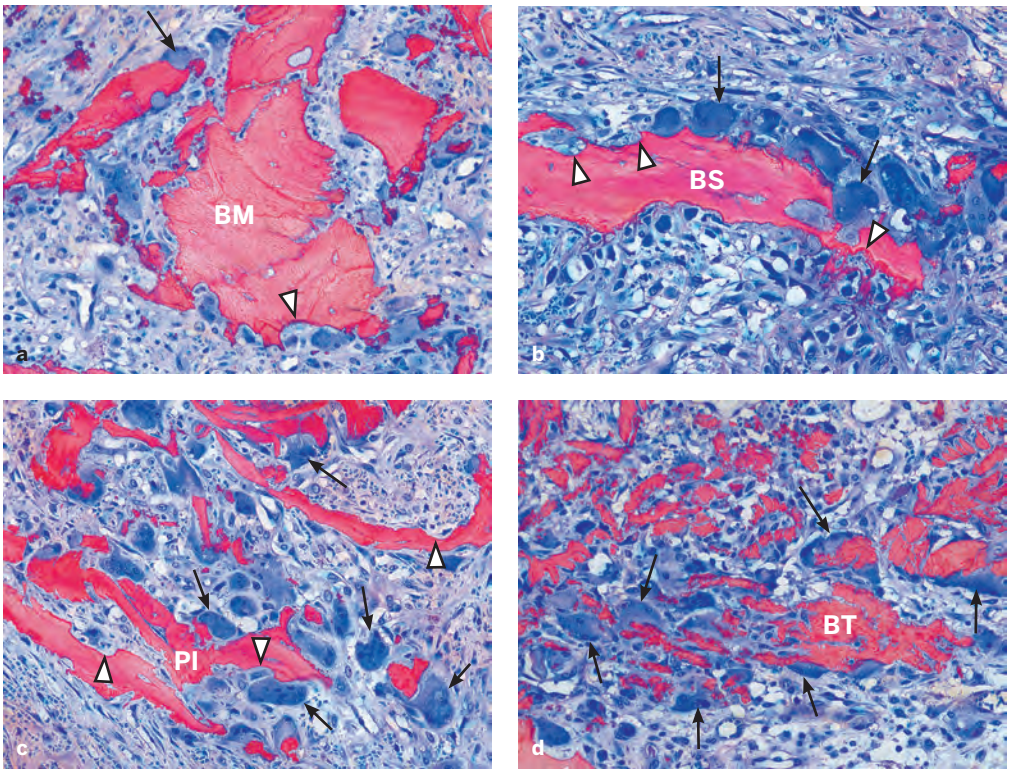


Fig 2-24 Histologic sections 1 week after implantation resorption of autogenous bone. *(a)* Particulate from corticocancellous bone with a bone mill (BM). *(b)* Bone chips harvested with a bone scraper (BS). *(c)* Bone particles harvested with a piezoelectric instrument (PI). *(d)* Bone slurry from a bone trap filter (BT). All harvesting techniques show numerous Howship lacunae *(arrowheads)* and osteoclasts *(arrows)*. The lowest number of osteoclasts is found on BM particles, and the highest number is found on BT particles.

Autogenous bone from bone mills. Particulate bone ground in a bone mill requires a bone block to be harvested from a donor site and involves an additional surgical intervention, causing morbidity. This procedure may provide more autograft volume than is available with bone dust from bone collectors. The milling process may reduce the number of viable cells.

Autogenous bone collected with a piezoelectric technique. There are not many data available on this harvesting technique. A recent preclinical study has shown that the dimensions of particulate autografts harvested with a piezoelectric device resemble those of bone chips collected with a bone scraper.[92]

Autogenous bone from bone scrapers. This harvesting technique has been advocated over the past few years for minor bone regeneration procedures, such as extraction sockets and localized sinus augmentation procedures, or coverage of dehiscence-type defects, either alone or in combination with bone substitute materials.[95–97] With this technique, small particles of cortical bone are harvested by scraping the bone surface—a simple intraoral approach with which up to 5 cm^3 of bone can be obtained.[95] Osteocytes have been shown to survive the grafting procedure, but because of the cortical nature of the graft, very few osteoblasts and osteoblast precursors are expected to be present. The resistance against resorption is presumably low because of the high surface area–to-volume ratio.[97]

Autografts are still considered the gold standard in osseous reconstructive surgery.[98] However, significant drawbacks related to the use of autografts have intensified the search for alternatives. First, there is

the unpredictable resorption of up to 60% of corticocancellous block grafts.[99] If uniform resorption could be expected, it would be unproblematic to perform a standardized overcompensation of the augmented volume. Second, there is donor site morbidity. This is most pronounced in relation to the extraoral donor sites,[100] but it may also be significant in intraoral grafting procedures.[89] Finally, it can be a problem that autogenous bone is not available in unlimited quantities. With the main goal of reducing or even eliminating the shortcomings of autografts, the search for appropriate bone substitute materials has been going on for the past 50 years.

Allografts

Allografts consist of bone obtained from a donor and used in a member of the same species. Transplantation of bone from one individual to another has been performed in orthopedic surgery for more than 130 years.[101]

Allografts are usually stored in bone banks, and they may be used as fresh frozen bone (FFB), freeze-dried bone allograft (FDBA), or demineralized freeze-dried bone allograft (DFDBA). FFB is rarely used in GBR procedures because of a high risk of immunologic rejection and disease transmission, whereas the freeze-drying of FDBA and DFDBA is reported to reduce the immunogenicity of the material, potentially improving the clinical outcome. Allografts are available as blocks or in particulate forms of both cortical and cancellous origin.[102,103]

FDBA and DFDBA have been shown to be biocompatible and to contain osteoinductive molecules such as BMPs.[104] Demineralization of allografts is intended to expose the BMPs, additionally increasing the immediate osteoinductive potential. However, FDBA loses some of its mechanical stability during the demineralization process, and DFDBA should therefore be used in combination with a space-maintaining material if the bone defects are not self-contained. Different batches of DFDBA have been shown to contain very different concentrations of BMPs,[105] and the osteoinductivity can therefore be expected to vary correspondingly.

Histologic evidence from an experimental comparative study in the mandibles of minipigs showed that allografts decelerated new bone formation in comparison with autografts (positive control) and coagulum (negative control).[106] DFDBA showed osteoconductive properties, but osteoinductive potential could not be demonstrated.[106] Therefore, FFB, FDBA, and DFDBA indisputably contain osteoinductive molecules. However, it is still debatable whether the concentrations of these BMPs are sufficient to elicit clinically relevant osteoinductive potential and whether they are present in an active form.

Allografts are widely used in the United States, whereas local regulations in Europe restrict the collection of human bone. This has limited their popularity among clinicians. Compared with the limitations of autografts, donor site morbidity is not an issue, and allografts are available in abundant quantities. However, resorption is reported to take place, as is seen with autografts.[102]

Xenografts

Xenografts, or xenogeneic bone substitutes, consist either of bone mineral derived from animals or bone-like minerals derived from calcifying corals or algae that have had the organic component removed to eliminate the risk of immunogenic reactions or transmission of diseases.

A few species of calcifying corals were found to have a calcium carbonate skeleton with a geometry similar to that of human cancellous bone, with interconnected macropores of 20 to 600 µm. The coralline calcium carbonate is transformed into hydroxyapatite (HA) by a hydrothermal exchange reaction with phosphorus. Experimental studies have demonstrated that the osteoconductive potential of coral-derived bone substitutes is less than that of other bone substitute materials.[106,107] Currently, coralline HA is seldom used for onlay grafts in GBR procedures because of a high rate of late complications.[108] When used as particulate, the granules tend to migrate, and the ones that are kept at the augmented site predominantly become encapsulated by fibrous tissue. The blocks, on the other hand, most often show bone formation throughout the augmented volume, but they are prone to develop late dehiscences.[109]

There is also a group of marine algae that consist of a calcified exoskeleton made of calcium carbonate. The natural material is converted into fluorohydroxyapatite through an exchange reaction with ammonium

phosphate at around 700°C. The morphologic structure is made up of pores arranged in parallel with a mean diameter of 10 µm and connected through microperforations. The pore configuration is thus not ideal for vascular ingrowth, but cellular invasion of the pores and bone deposition directly on the material surface have been documented.[110,111] Neovascularization is instead expected to take place in between the bone substitute particles. In contrast to coralline HAs, phycogenic fluorapatite undergoes slow resorption by enzymatic and cellular degradation, but at a lower rate than autografts.[110]

Most xenografts originate from natural bone sources in animals. In particular, cancellous bovine bone has been used as a source for these bone substitute materials because of its close similarity to cancellous human bone. The organic component is removed by heat treatment, by a chemical extraction method, or by a combination of the two, in order to eliminate the risk of immunologic reactions and disease transmission. Since the first reports of bovine spongiform encephalopathy, there has been a particular focus on the ability of these extraction methods to completely eliminate all proteins from the bovine bone source.[112,113] However, despite the hypothetical risk of organic remnants in bovine bone substitutes, there have been no reports of disease transmission with the use of these biomaterials. In contrast, a few cases of transmission of HIV and hepatitis related to allogeneic materials have been reported.[114]

Bovine-derived xenografts are generally biocompatible and osteoconductive, although the production methods have a strong impact on their biologic behavior. For instance, high-temperature treatment resulted in less bone formation and lower osteoconductivity.[107,111,115] This difference most likely reflects the production-related changes in surface characteristics. Temperatures exceeding 1000°C cause a sintering of the natural hydroxyapatite, with the apatite crystals growing and the intercrystalline spaces disappearing to a large extent.[116] This reduces the microroughness and micro- and nanoporosity of the bone substitute and increases the crystallinity.

Alloplastic bone substitutes

Alloplastic or synthetic bone substitutes have the advantage of not bearing any risk of disease transmission and are available in large quantities. Furthermore, it is theoretically possible to specifically design these biomaterials with material characteristics that meet the requirements for a specific clinical indication. Chemistry, phase distribution between crystalline and amorphous material, crystal size, gross morphology and size, particle size, pore size and interconnectivity, macro- and nanoporosity, and surface roughness and texture at the macroscale and nanoscale can be tailored. A greater understanding of the importance of the different material characteristics has helped us to understand why materials with identical chemical compositions and macromorphologies have performed so different biologically in vivo.

The synthetic biomaterials currently on the market can be divided into three groups: calcium phosphates (CaPs), bioactive glasses, and polymers (see Fig 2-22). Of these, CaPs—and especially HA and tricalcium phosphate (TCP)—have been most intensively studied due to their composition, which closely resembles the inorganic phase of bone.[117] In general, HA is considered to be osteoconductive and nonresorbable, whereas TCP also demonstrates osteoconductive properties but resorbs rapidly. Therefore, combinations of HA and TCP—biphasic calcium phosphates (BCPs)—have been investigated with the goal of benefitting from both the stable space-keeping properties of HA and the resorbable space-making properties of TCP.[118] By varying the HA:TCP ratio, it was possible to modulate the substitution rate and bioactivity of these biomaterials.[119,120] BCPs are a valuable alternative for clinicians and patients who are not comfortable with implantation of material of human or animal origin. Future perspectives may involve the development of a selection of two to three BCPs to fit individual clinical indications. A very interesting and promising research field is the development of biomaterials, particularly BCPs, that are osteoinductive. This biomaterial-induced or intrinsic osteoinduction should not be confused with the biomaterial-free induction of undifferentiated inducible osteoprogenitor cells.[121] Thus far, this intrinsic, biomaterial-dependent osteoinduction is considered unpredictable in various animal models and in humans. A mechanism for biomaterial-induced heterotopic ossification has been proposed, for example by Bohner and Miron.[121]

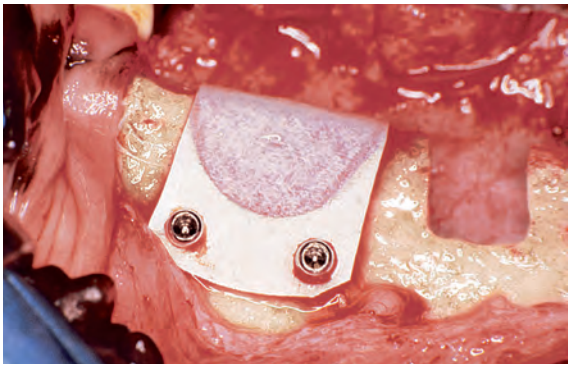


Fig 2-25 Surgical procedure of GBR. Two defects were created in the exposed mandible of a dog 2 months after tooth extractions. The right defect is open, but the left one is covered with a nonresorbable ePTFE membrane. Miniscrews fix the membrane and mark the corners of the defect for radiography.

Bioglasses are silica-based materials that were first introduced in the early 1970s. These glasses exhibit bone bonding as a result of the surface reactive silica, calcium, and phosphate groups that are characteristic of these materials. Silica is believed to play a critical role in bioactivity. Bioactive glasses are very biocompatible materials. Some experimental data support their use in GBR procedures such as ridge preservation and sinus augmentation. However, there are inherent limitations on the currently available bioglass products. Because of their granular and nonporous nature, they cannot reliably serve as space-maintaining devices, although macroporous glass ceramics are now also available.[122]

Bone healing in membrane-protected defects without addition of a bone filler

The histologic healing pattern of bone regeneration under a barrier membrane without addition of a bone filler was demonstrated by Schenk et al in a landmark experimental study.[123] Saddle-type defects were created in the mandibles of dogs 2 months after tooth extractions. The defects were covered with (test) or without (control) a barrier membrane (Fig 2-25). A standard ePTFE membrane and two different prototype ePTFE membranes reinforced with polypropylene mesh were used. The position of the membranes was secured by two miniscrews. No bone filler was used. However, intravenously aspirated blood was injected under each membrane. Histologic analysis was performed after healing periods of 2 and 4 months.

Healing without a barrier membrane

The control defects showed a consistent repair pattern in which bone formation was restricted to the defect margins, ie, mesial and distal walls of the defect and at the bottom of the defect (Fig 2-26a). Closure of the marrow space was completed after 2 months, but bone formation made no further progress. At 4 months, bone had slightly increased in density.

Healing under a barrier membrane

Membrane protection resulted in a dramatic change in bone regeneration. The membrane maintained the space created during surgery and clearly separated the outer compartment, constituting the oral mucosa, from the inner space, which was mainly accessible from the marrow cavity (Fig 2-26b). This inner compartment was initially filled with a blood clot, and at 2 months, remnants of the coagulum could still be recognized in the middle portion of the defect (Figs 2-27 and 2-28).[123] However, the hematoma was completely penetrated by granulation tissue and blood vessels. The majority of the secluded volume now consisted of a spongeous bony regenerate that enclosed, between its trabeculae, a labyrinth of tiny, interdigitating marrow spaces filled with hypervascularized, loose, soft connective tissue. Both the vessels and the fibrous tissue were in continuity with the original bone marrow at the bottom of the defect. Bone formation started, as in the controls, from the margins of the defects, where it spread over the openings of the marrow cavity (see Fig 2-28).

Thus, there were basically three centers of bone formation, and these formed a dome-shaped seal over

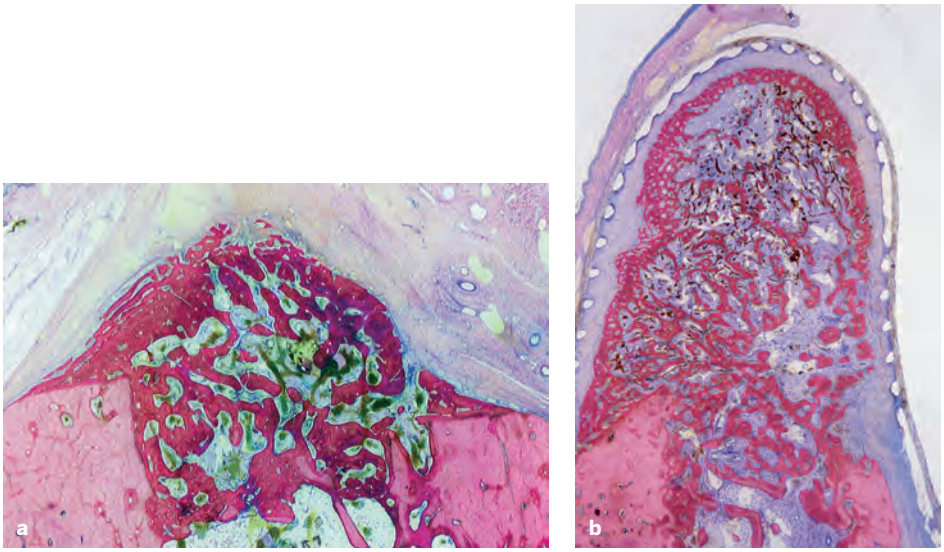


Fig 2-26 Buccolingual ground sections through the central portion of *(a)* a control site (without membrane) and *(b)* a test site (with a nonresorbable ePTFE membrane) after 2 months. While a low bony cover seals off the marrow space at the bottom of the control defect, the regenerated bone completely fills the secluded space beneath the barrier membrane at the test site (undecalcified ground section; toluidine blue and basic fuchsin stain).

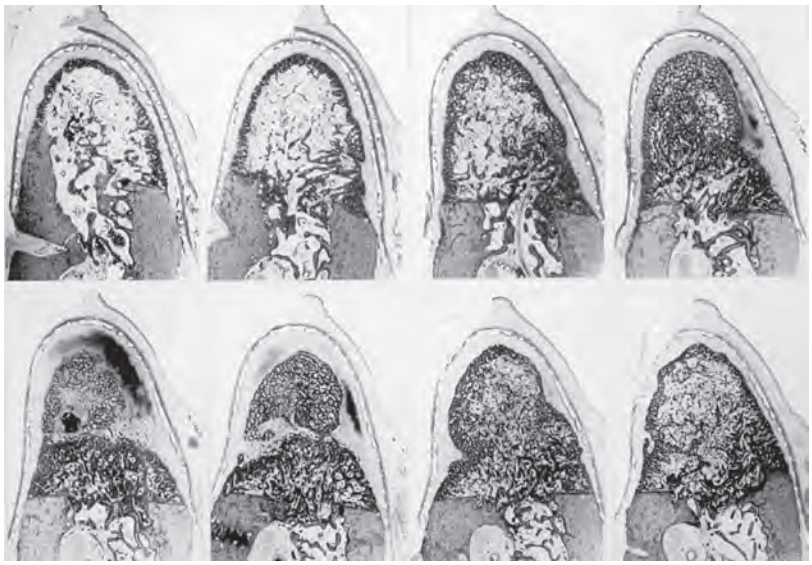


Fig 2-27 Selected serial buccolingual sections, arranged in a mesiodistal sequence, of a membrane-covered defect after 2 months. (Reprinted with permission from Schenk et al.[123])

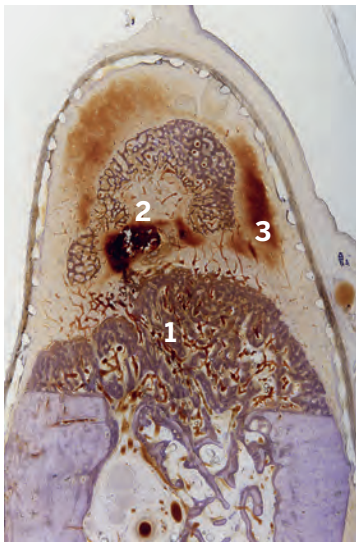


Fig 2-28 The fifth section from Fig 2-27 to show (1) the bony cover of the bottom marrow space and (2) a tangential section through the top of the bony hill originating from the mesial wall of the defect, as well as (3) the associated hematoma.

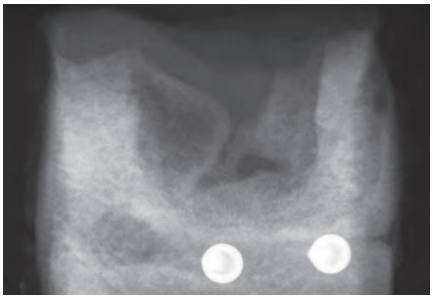


Fig 2-29 Radiograph of a membrane-covered defect after 2 months shows bony ingrowth from the mesial and distal walls, as well as from the bottom of the defect. (Reproduced with permission from Schenk et al.[123])

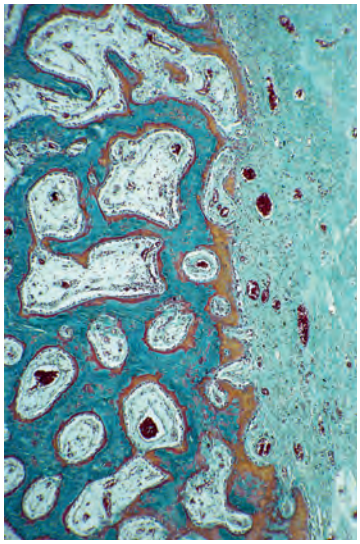


Fig 2-30 Organization of the hematoma and woven bone formation. Blood vessels and bone-forming cells invade the former hematoma *(right)* and construct a scaffold of woven bone (Goldner trichrome stain).

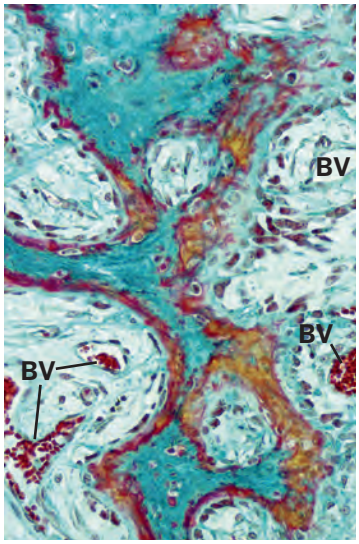


Fig 2-31 In the advancing ossification front, blood vessels (BV) and outgrowing trabeculae are tightly interconnected. Goldner trichrome stain stains osteoid seams in *red* and mineralized bone matrix in *green*.

the openings of the marrow cavity. From these bony covers, bone further expanded into the center of the membrane-bound space. Both radiographs (Fig 2-29) and serial ground sections (see Fig 2-27) demonstrated this characteristic pattern of bone healing.[123] See Fig 2-28 for an illustration of the third center of bone formation at the bottom of the defect.

Formation of the primary spongeous scaffold. The histology of bone formation in the membrane-protected space exhibited a remarkable similarity to that found during bone development and growth (Fig 2-30). The infiltration of the hematoma by granulation tissue followed the basic pattern of wound healing. The invading vascular sprouts were accompanied by cells originating from the bone marrow at the periphery of the defect, thus enabling mesenchymal stem cells to differentiate into osteoblasts.[124] From the cut cortical and trabecular surfaces, woven bone sprouted out, mostly in the shape of thin, bifurcating plates (Fig 2-31). A particular characteristic of this primary cancellous scaffold is the perfect interdigitation with the vascular plexus. As already mentioned, angiogenesis and an ample blood supply are mandatory for bone development and maintenance.

Transformation into compact bone and a regular spongiosa. While the original primary trabeculae consisted exclusively of woven bone, it later served as a template for the apposition of parallel-fibered bone followed by lamellar bone, which eventually would constitute both compact bone and a regular spongiosa with mature bone marrow. These events occurred 3 to 4 months after surgery (Fig 2-32).

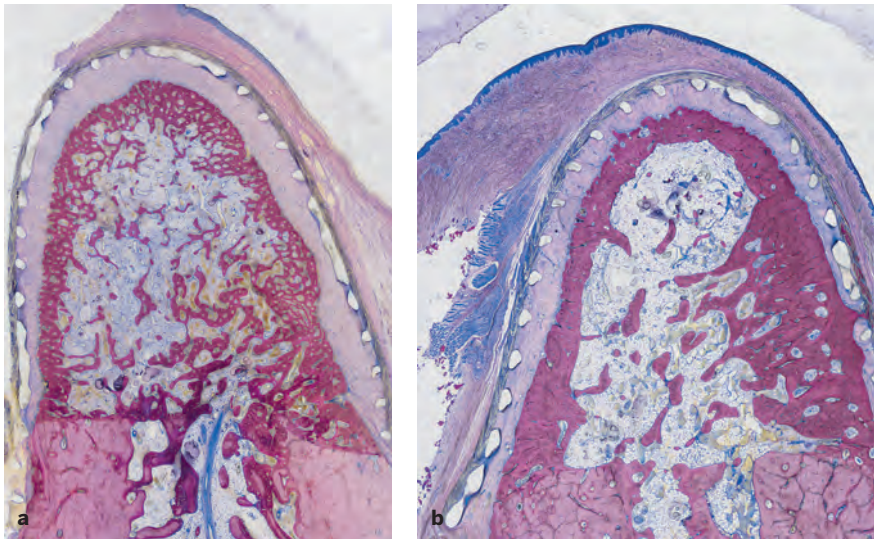


Fig 2-32 Transformation of the primary spongiosa spongework into cortical bone and cancellous bone. *(a)* After 2 months, the spongiosa is denser at the periphery than in the center of the bony regenerate. *(b)* Cortical bone and secondary spongiosa after 4 months. A compact bone layer in the periphery confines a cancellous bone in the center with well-defined trabeculae and regular bone marrow (surface staining with toluidine blue and basic fuchsin).

Remodeling of cortical bone. During the fourth month, the cortical bone entered its last phase of maturation: Haversian remodeling.

Modeling of the bony regenerate. At the end of the fourth month, growth and modeling of the bone within the membrane-confined space continued, particularly in the central portion. With the formation of a cortical bone layer, the periosteal and endosteal envelopes were also restored. As long as modeling was occurring, the bone surface was locally lined by osteoblasts and an osteoid seam, or was covered by osteoclasts or Howship lacunae, as a sign of ongoing or past resorptive activity.

Bone healing in membrane-protected defects with addition of a bone filler

The healing pattern of bone under a barrier membrane with the addition of various particulate bone fillers was analyzed in numerous experimental animal studies. One standardized animal model was introduced by Buser et al.[106] In this model, which excludes the interference of the particular conditions in the oral cavity, standardized bone defects were created in the mandibles of minipigs with extraoral access. This study showed that particulate bone fillers have different biologic characteristics with regard to both bone formation potential and bone filler degradation dynamics. After 4 weeks, which was the earliest observation period in this study, significantly more new bone was formed when autogenous bone was used as a filler, as compared to DFDBA, a synthetic β-TCP biomaterial, and coral-derived HA (Fig 2-33).[106] After 12 and 24 weeks, there was still more bone formation in the autogenous bone group than in the groups with DFDBA and coral-derived HA, but most new bone was found in the TCP group. On the other hand, TCP showed the greatest degradation rate, meaning that the volume of all three other fillers was more stable. Another important finding was that the autograft was the most osteoconductive filler material over the entire observation period (Fig 2-34).[106] From this study, it was concluded that defects filled with autograft clearly demonstrated the best results in the early phase of healing and that the TCP biomaterial used showed a fast degradation and substitution rate.

Based on these intriguing results, earlier observation periods down to 1 week were chosen, and other bone filler biomaterials were tested in subsequent studies.[119,120,125,126] Collectively, these studies confirmed that the autograft caused most new bone formation—at least up to 4 weeks (Figs 2-35 to 2-37)—and was the most osteoconductive filler material (Fig 2-38). It was also confirmed that the β-TCP biomaterial lagged behind but was catching

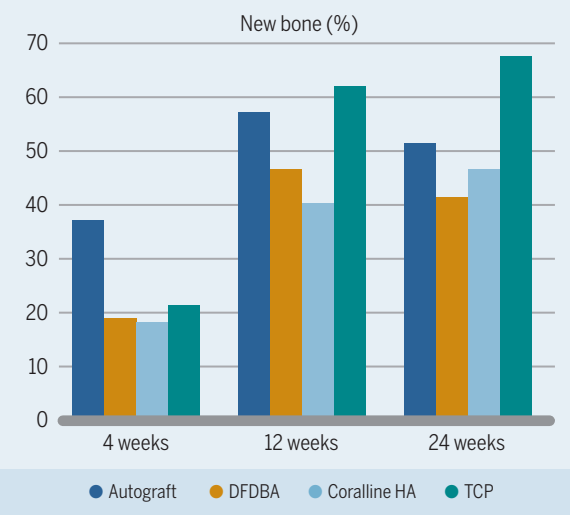


Fig 2-33 Percentage of new bone in standardized bone defects in the mandibles of minipigs grafted with different materials. (Data from Buser et al.[106])

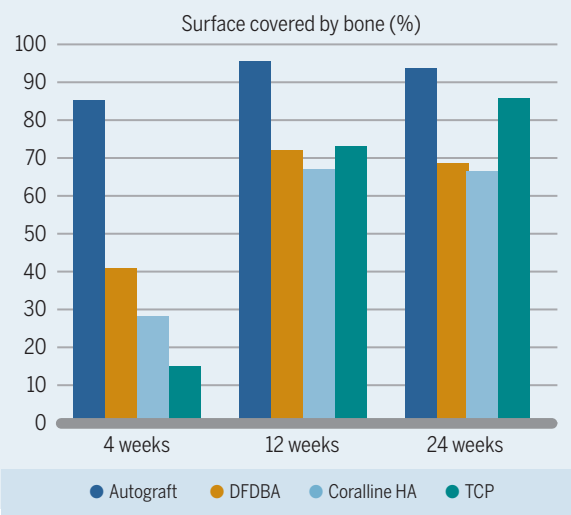


Fig 2-34 Percentage of grafting material surface covered with bone as an indicator of the osteoconductive potential of different grafting materials in standardized bone defects in the mandibles of minipigs. (Data from Buser et al.[106])

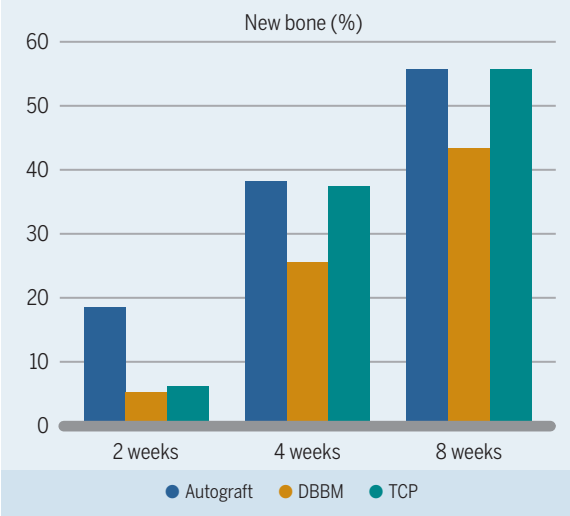


Fig 2-35 Percentage of new bone in standardized bone defects in the mandibles of minipigs after grafting with different materials. (Data from Jensen et al.[125])

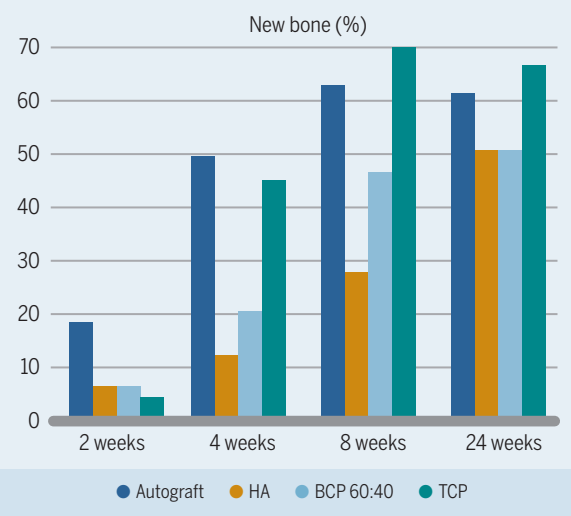


Fig 2-36 Percentage of new bone in standardized bone defects in the mandibles of minipigs after grafting with different materials. The three bone substitute materials have identical material characteristics except for the chemical composition. In the early healing phases, more new bone formation is seen in defects grafted with TCP than with BCP, which resulted in more new bone formation than HA. (Data from Jensen et al.[119])

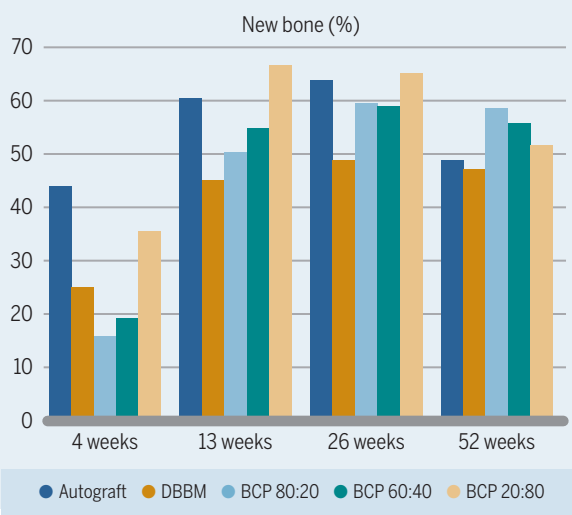


Fig 2-37 Percentage of bone formation in standardized bone defects in the mandibles of minipigs after grafting. In the early healing phases, more new bone formation is seen in defects grafted with BCPs with high TCP content. (Data from Jensen et al.[120])

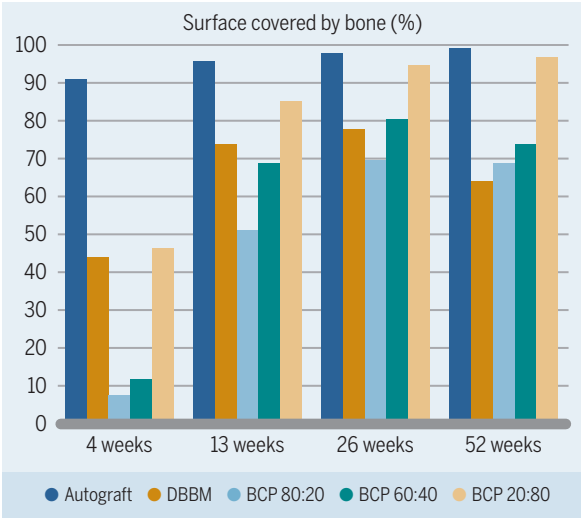


Fig 2-38 Percentage of grafting material surface covered with new bone in standardized bone defects in the mandibles of minipigs. (Data from Jensen et al.[120])

up with the autograft group. Furthermore, these studies demonstrated that use of synthetic β-TCP led to faster bone formation than use of synthetic HA, and that biphasic BCP with a 60:40 ratio of HA and TCP was somewhere in between (see Fig 2-36). Varying the ratio of HA and TCP showed that the higher the TCP content was, the more quickly bone formation occurred (see Fig 2-37).

What has not been mentioned thus far is the HA of bovine origin. Various brands of bovine-derived bone substitutes are available on the market. However, one of them outclasses all other forms in terms of quantity of scientific data: Bio-Oss (Geistlich), also known as deproteinized bovine bone mineral (DBBM), bovine-derived bone mineral (BDBM), or anorganic bovine bone (ABB). Bio-Oss has been commercially available for regenerative dentistry for more than 30 years.

Some controversy remains as to whether DBBM is truly resorbable.[127] It has been shown in vitro that osteoclast progenitor cells are able to proliferate on DBBM surfaces, and later, as osteoclast-like cells, produce resorption pits. Compared with native bovine bone, however, the osteoclasts are reduced in number and size, and the resorption pits are less pronounced.[115] Experimental in vivo studies have also demonstrated multinucleated giant cells on DBBM surfaces that stain positive for TRAP[119,120,128–130] and occasionally display a sealing zone, a ruffled border, and shallow Howship lacunae[130] (Fig 2-39). These findings suggest that these cells have osteoclast-like properties, but fail to resorb this biomaterial, at least at a fast pace. Also, in the mandibular defect model in the minipig, a significant reduction in volume over observation periods of up to 1 year was not seen.[120] Human biopsies after sinus augmentation confirm that particles of DBBM can still be found up to 20 years postoperatively[131] (Fig 2-40). Thus, in daily practice, this particular xenograft must be considered very resistant to resorption.

In the studies by Jensen et al[120,125] and Broggini et al,[126] the amount of new bone in defects filled with DBBM increased over time, yet at a slower pace than around the autograft particles (see Figs 2-35 and 2-37). Likewise, osteoconductivity was lower with DBBM than with autograft particles (see Fig 2-38). This difference was clearly visible for up to 4 weeks after defect fill, and most pronounced after 1 to 2 weeks (Fig 2-41). Although bone regeneration started from the defect margins for all types of bone fillers,

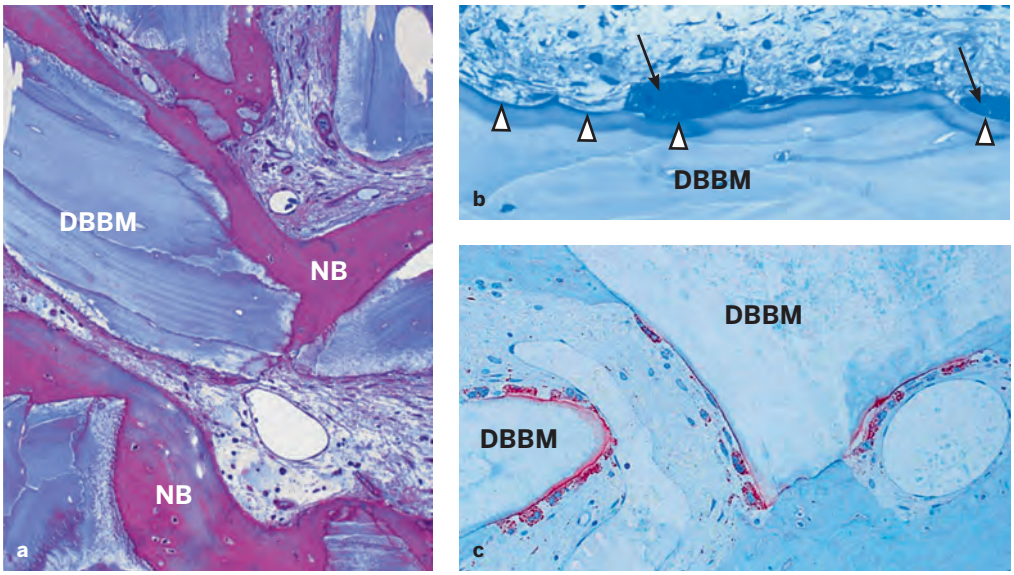
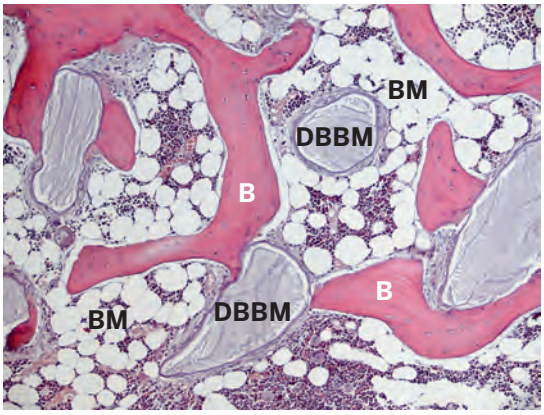


Fig 2-39 Histologic sections of human biopsy specimens grafted with DBBM (thin sections of decalcified tissue). *(a)* The DBBM particles show good tissue integration. Newly formed bone (NB) covers part of the bone substitute surface and bridges neighboring DBBM particles (basic fuchsin and toluidine blue stain). *(b)* Osteoclast-like, multinucleated giant cells *(arrows)* are frequently seen lining the DBBM surface, which occasionally shows shallow resorption depressions *(arrowheads;* toluidine blue stain alone). *(c)* Staining for tartrate-resistant acid phosphatase (TRAP; *red staining)* identifies osteoclast-like, multinucleated giant cells on the surface of the DBBM particles (stained for TRAP and counterstained with toluidine blue).

Fig 2-40 Histologic section of a human biopsy specimen harvested 20 years after sinus floor elevation with DBBM. DBBM particles are interconnected by bone matrix (B) and surrounded by mature fatty bone marrow (BM; thin paraffin section of decalcified tissue; toluidine blue and basic fuchsin stain).



progression of bone formation and consequently defect fill with new bone was fastest when autogenous bone chips were used (Fig 2-42).

These and other studies came to the conclusion that it would be advantageous to combine the best of two worlds: autogenous bone, with its excellent osteopromotive and osteoconductive properties, boosting bone formation in the early healing phase; and DBBM, with its low degradation rate, helping to keep the gained bone volume stable over the long term. Indeed, clinical and radiographic data from humans[132–135] have confirmed this biology-inspired concept, in which autogenous bone chips and DBBM are placed layer by layer to augment peri-implant bone defects. In this two-layer approach, the autograft particles are placed close to the implant surface to promote bone outgrowth from preexisting bone, and the DBBM particles form an external shield against resorption.

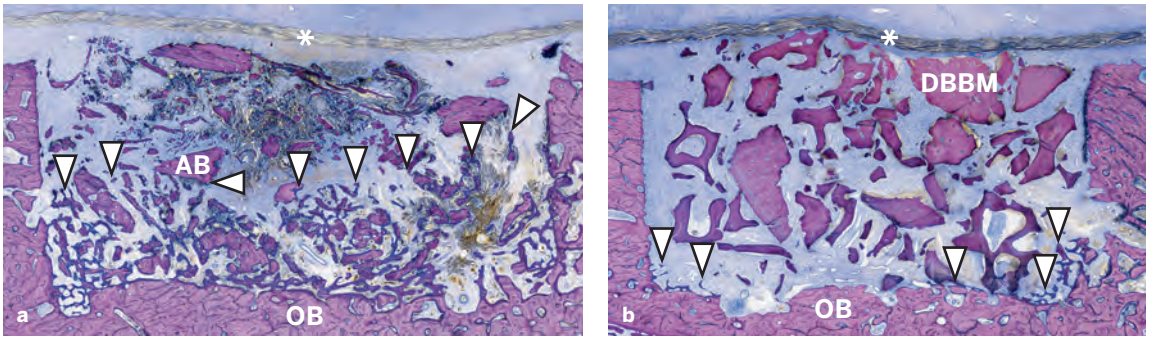


Fig 2-41 Histologic sections of standardized bone defects in the minipig mandible 2 weeks after GBR with an ePTFE membrane *(asterisks)* and autogenous bone chips (AB) *(a)* or DBBM *(b)* (undecalcified ground sections; toluidine blue and basic fuchsin stain). The defect margins are delineated by old bone (OB) and the membrane. Formation of new bone *(arrowheads)* begins from the defect margins. New bone formation is much more advanced *(arrowheads)* in defects filled with autogenous bone than with DBBM.

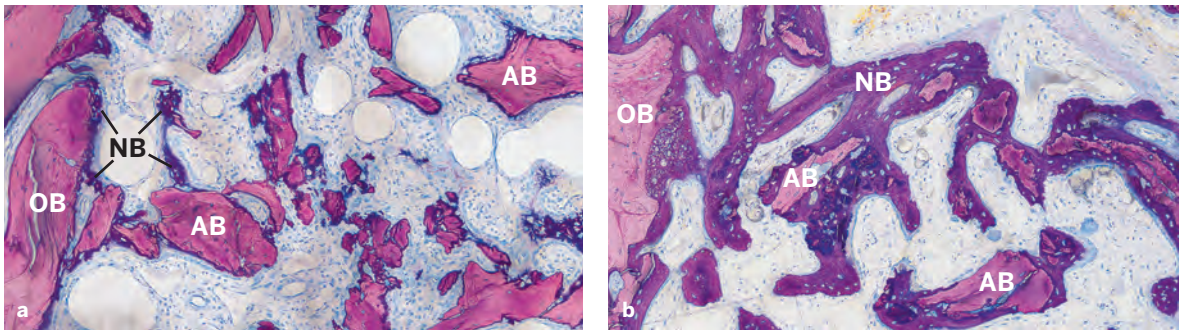


Fig 2-42 Histologic sections of standardized bone defects in the minipig mandible 1 and 2 weeks after GBR with an ePTFE membrane and autogenous bone chips (AB; undecalcified ground sections; toluidine blue and basic fuchsin stain). Formation of new bone (NB) begins from the surface of old bone (OB) at the defect margins and progresses toward the center of the defect. New bone formation is already visible after 1 week *(a)* and much more advanced after 2 weeks *(b)*.

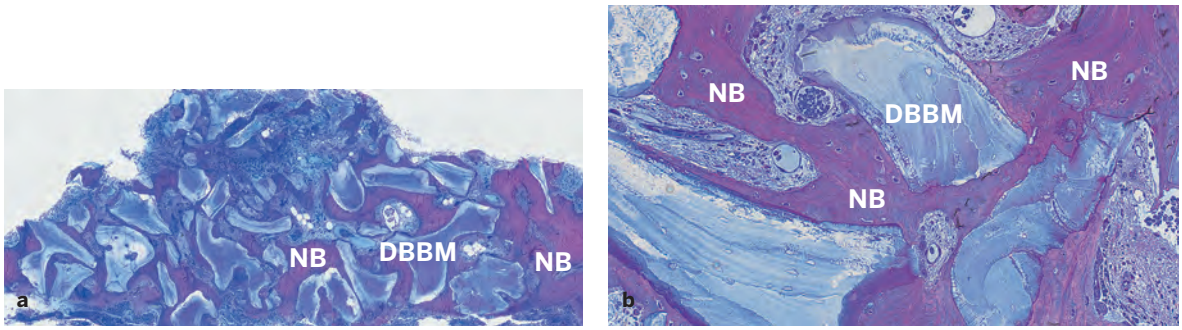


Fig 2-43 Histologic section of a human biopsy specimen harvested 74 months after contour augmentation lateral to a dental implant with particulate autogenous bone, DBBM, and a collagen membrane (decalcified tissue; toluidine blue and basic fuchsin stain). Low *(a)* and high *(b)* magnifications demonstrate good tissue integration of DBBM particles. Newly formed bone (NB) covers a substantial portion of the DBBM surfaces and bridges neighboring DBBM particles. Adipocytes indicate presence of mature bone marrow.

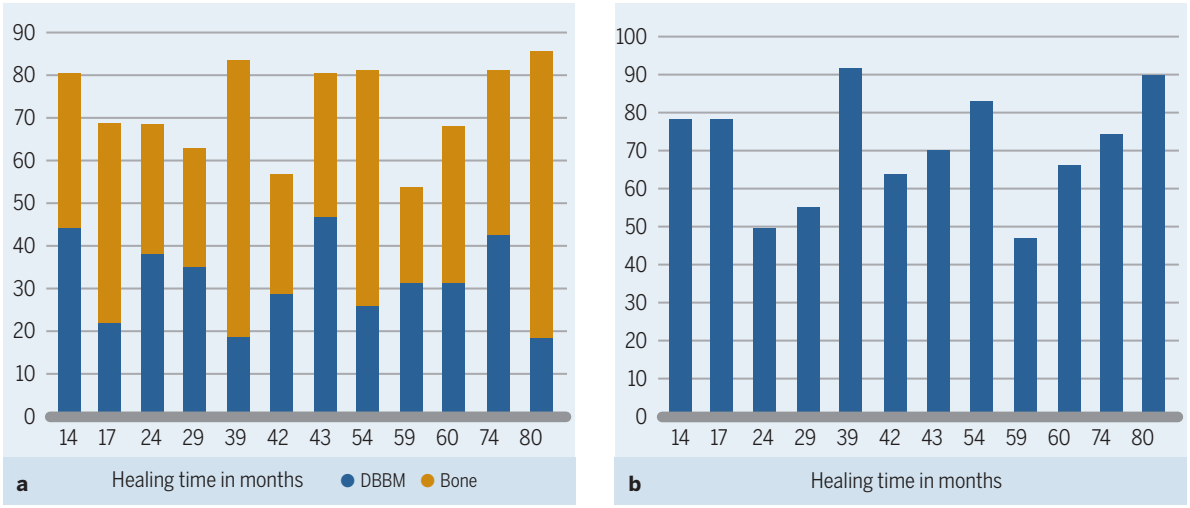
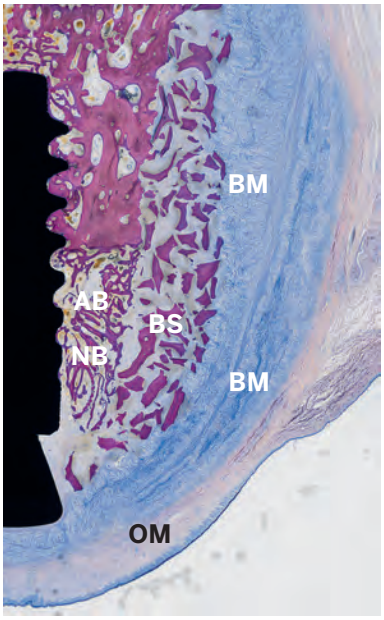


Fig 2-44 Histograms for 12 human biopsy specimens harvested from 14 to 80 months after contour augmentation with particulate autologous bone, DBBM, and a collagen membrane. *(a)* Area fractions of new bone and DBBM. *(b)* Percentage of DBBM surface covered with new bone.

Fig 2-45 Light micrograph demonstrating perfect structural integrity of an augmented site 3 weeks after contour augmentation with autogenous bone chips (AB), a bone substitute (BS; Bio-Oss), and a non-cross-linked barrier membrane (BM; Bio-Gide) of a buccal peri-implant bone defect in a dog mandible. Residues of the double-layered barrier membrane are still clearly visible, and the oral mucosa (OM) is blocked off. New bone (NB) has formed in the inner portion of the augmented region (undecalcified ground section; toluidine blue and basic fuchsin stain).



Tiny biopsies harvested 14 to 80 months after contour augmentation in patients supplied histologic evidence of long-term stability when applying this concept.[129] While the histology has shown good integration of DBBM in newly formed bone (Fig 2-43), histomorphometry has demonstrated stable new bone volume and no signs of significant substitution of DBBM particles over time (Fig 2-44). Thus, earlier findings from preclinical studies were confirmed, and it was suggested that the low substitution rate of DBBM may be the reason for the clinically and radiographically documented long-term stability of contour augmentation, when a combination of autogenous bone chips, DBBM particles, and a collagen membrane is used. In a recent preclinical study, it was shown that the addition of autogenous bone chips and the presence of a collagen membrane increased bone formation around DBBM particles[136] (Figs 2-45 and 2-46).

Fig 2-46 Relative amounts of osteoid, new mineralized bone, DBBM, bone autograft, and soft tissue in the grafted region 3 and 12 weeks after contour augmentation in dog mandibles. Four combinations of 3 different materials were tested: (A) autogenous bone chips, (D) DBBM, and (M) collagen membrane. The addition of autogenous bone chips and the presence of the collagen membrane increased bone formation around DBBM particles.

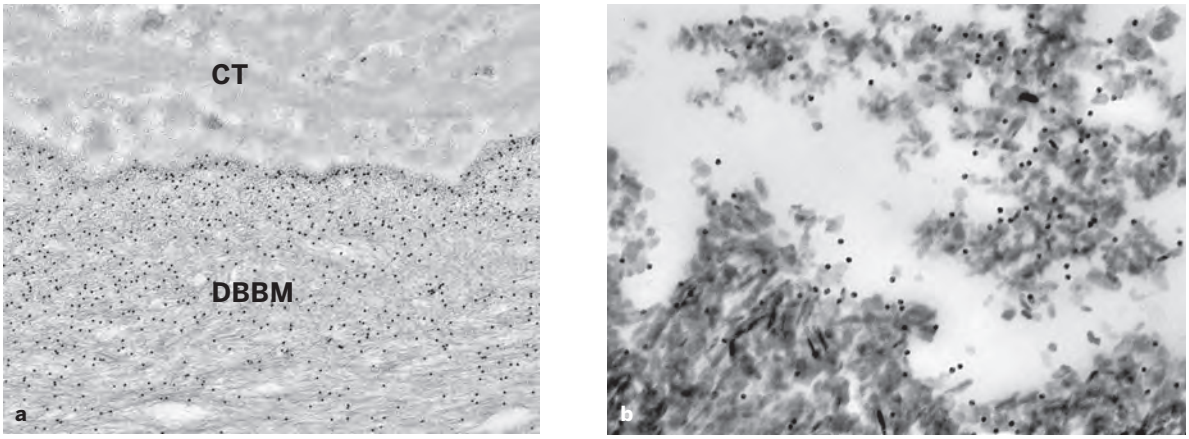


Fig 2-47 *(a)* Ultrathin section of a human biopsy specimen retrieved from a bony site augmented with DBBM and embedded in acrylic resin. High-resolution postembedding immunocytochemistry with an antibody against a typical bone-related noncollagenous protein demonstrates gold particle *(black dots)* labeling preferentially at the periphery of the DBBM particle. This finding indicates that DBBM takes up the patient's own proteins from the wound environment after implantation. *(b)* High-resolution postembedding immunocytochemistry with an antibody against enamel matrix proteins demonstrates the ability of DBBM to be precoated with biologically active molecules prior to implantation. CT, soft connective tissue.

Have we reached the limit with the contour augmentation technique using GBR? As we have seen, bone formation around bone substitutes lags behind bone formation around autogenous bone chips during early healing periods. Thus, it is tempting to find ways to improve the performance of bone substitutes. One possibility is to coat them with biologics like growth factors, a process called *biofunctionalization*. In particular, DBBM appears to be very suitable for this, because its high macro- and nanoporosity can absorb a lot of the patient's own proteins and other macromolecules from the environment (Fig 2-47a) and can be precoated with biologically active molecules[137,138] (Fig 2-47b). In chapter 3 of this book, the scientific background and value of biofunctionalizing biomaterials with bone-conditioned medium (BCM)—ie, the patient's own growth factors released from bone—is discussed in detail.

Conclusion

Bone has the unique capability to rebuild its original structure and function in response to a trauma. The trick in GBR is to harness this great regenerative potential to enhance bone formation around dental implants or at sites destined for implant placement. Under stable mechanical conditions, bone is formed directly or primarily, provided that two essential conditions are present: an ample blood supply and a solid base for bone deposition. The solid base is provided by the bony margins of a defect. Bone healing around dental implants is special in the sense that the implant surface in the defect region is devoid of bone, and thus bone has to grow into the defect from the walls of the preexisting bone. While the function of the barrier membrane is to keep away unwanted, fast-growing tissues, the functions of bone fillers are manifold. Because one type of bone filler cannot fulfill all requirements, the best of two worlds should be combined: autogenous bone with its great osteoconductive, osteoinductive, and osteogenic potential, boosting bone formation in the early healing phase; and a low-substitution filler that keeps the gained bone volume stable in the long term.

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